Induction of Endothelial Cell Expression of the Plasminogen Activator Inhibitor Type 1 Gene by Thrombosis In Vivo

Satoshi Fujii, MD, PhD; Hiroyuki Sawa, MD; Jeffrey E. Saffitz, MD, PhD; Charles L. Lucore, MD; and Burton E. Sobel, MD

Background. We have shown previously that products from activated platelets can augment synthesis of plasminogen activator inhibitor type 1 (PAI-1) in cultured endothelial and hepatoma (Hep G2) cells in vitro and increase plasma PAI-1 activity in vivo in rabbits. Accordingly, the effects of activation of platelets associated with thrombosis and thrombolysis in vivo on plasma PAI-1 activity and expression of the PAI-1 gene in endothelium, liver, and other organs were characterized.

Methods and Results. Endothelial injury giving rise to platelet-rich thrombi was induced with electrical stimulation in carotid arteries in rabbits. Clot lysis and recanalization were induced subsequently with intravenous tissue-type plasminogen activator (t-PA) and verified with Doppler flow probes. Plasma PAI-1 activity (mean±SD) increased from 6±2 arbitrary units (AU)/ml to 129±48 AU/ml (n=15) within several hours after recanalization. When t-PA had failed to induce recanalization, the increase was much less (from 7±2 to 42±23 AU/ml, n=11). To define mechanisms responsible for these changes, PAI-1 messenger RNA (mRNA) was evaluated by Northern blot analysis and localized in tissues by in situ hybridization. Strong and consistent induction of PAI-1 mRNA was evident in aorta, heart, and liver of animals subjected to thrombosis (twofold to threefold increases compared with values in controls), particularly in those in which thrombosis had been induced (fourfold to sixfold). After thrombolysis, an intense, PAI-1 mRNA-specific signal was detected in endothelium of aorta, liver, and heart, with less intense signals in endothelium of lung, adrenals, and kidneys.

Conclusions. The increases in plasma PAI-1 activity follow a preceding increase in endothelial cell expression of the PAI-1 gene as reflected by PAI-1 mRNA levels. Thus, increased synthesis of endothelial cell PAI-1 after thrombosis and thrombolysis may attenuate endogenous fibrinolysis early after coronary thrombosis, thereby potentiating early, thrombotic reocclusion. (Circulation 1992;86:2000–2010)

Key Words • thrombosis • plasminogen activator • inhibitor type 1 • endothelium • PAI-1 gene expression

Therapeutic benefits of coronary thrombolysis are unavoidably limited to some extent by initial failure of recanalization and early thrombotic reocclusion.1 Activation of platelets appears to contribute to both.2–5 We have shown previously that products of platelet activation can increase the synthesis of plasminogen activator inhibitor type 1 (PAI-1), the primary physiological inhibitor of tissue-type plasminogen activator (t-PA), both in vitro and in vivo6–8; that plasma PAI-1 activity is increased after acute myocardial infarction in patients9; and that activation of platelets in association with thrombosis and thrombolysis can augment plasma PAI-1 activity in vivo.10 However, the underlying changes in synthesis or degradation of PAI-1 in tissues responsible for these phenomena in vivo have not yet been elucidated.

PAI-1 biosynthesis occurs in endothelial and hepatoma (Hep G2) cells in culture.11 The present study was designed to determine whether endothelium, liver, and other tissues assayed by Northern blotting and in situ hybridization manifest changes in PAI-1 gene expression in vivo reflected by PAI-1 messenger RNA (mRNA) levels after thrombosis and thrombolysis and if so, whether the changes precede and therefore may account for subsequent changes in plasma PAI-1 activity elicited by thrombosis and thrombolysis. The results demonstrate that expression of the PAI-1 gene is stimulated markedly by thrombosis and thrombolysis resulting in striking increases in PAI-1 mRNA in liver, heart, and aorta, primarily in the endothelium of each.

Methods

Experimental Procedures

Animal preparations. Protocols used conformed to the "Position of the American Heart Association on Research Animal Use" (November 11, 1984) and were

From the Cardiovascular Division, Washington University, St. Louis, Mo.
Supported in part by National Heart, Lung, and Blood Institute grant HL-17646, SCOR in Coronary and Vascular Diseases, and by a grant-in-aid from the American Heart Association, Missouri Affiliate (S.F.);
Address for correspondence: Satoshi Fujii, MD, Cardiovascular Division, Washington University School of Medicine, 660 South Euclid Avenue, Box 8086, St. Louis, MO 63110.
Received February 27, 1992; revision accepted September 9, 1992.
approved by the animal studies committee at Washington University. New Zealand White rabbits weighing 3.15–4.60 kg (Doe Valley Farms, Bentonville, Ark.) were anesthetized with 20 mg/kg ketamine i.m. (Ketalar, Parke-Davis, Morris Plains, N.J.) and 8 mg/kg xylazine (Rompun, Mobay, Shawnee, Kan.), intubated, and maintained in an anesthetized state with 0.5–0.8% isoflurane (AErrane, Anaquest, Madison, Wis.), an agent that does not abolish platelet-mediated cyclic flow variations after endothelial injury.12 Catheters were placed in both femoral arteries and veins for infusion of agents and withdrawal of blood samples. Serial samples (2 ml) were obtained in plastic syringes with a two-syringe technique, transferred immediately to tubes containing 12.9 mM of sodium citrate at 0–4°C, and centrifuged at 2,000g for 10 minutes. Sampled blood volumes were replaced with equal volumes of saline. Plasma samples were separated, frozen immediately in liquid nitrogen, and stored at −70°C.

For induction of thrombosis in vivo, the right carotid artery and the left jugular vein were exposed. An arteriovenous shunt was constructed between the two so that cerebral infarction could be precluded when thrombi were induced subsequently in the carotid artery and lysed as previously described.10

Induction of thrombosis. After a Doppler flow probe had been placed in the carotid artery, a plastic tube 4 mm long was applied around the vessel. The internal diameter of the tube was narrowed to produce a decrease of flow of 40%. Thrombosis was induced secondarily to electrical stimulation and injury of endothelium with current via a needle electrode as previously described.10 In brief, a 23-gauge needle electrode was inserted obliquely into the lumen of the artery distal to the Doppler probe and proximal to the plastic tubing. It was stabilized with sutures through the extravascular tissue on either side of the artery. Electrical stimulation was initiated by connecting the electrode in series with the positive terminal of a 9-V battery, an ammeter, and a 50-kΩ potentiometer. A ground wire was sutured to the subcutaneous tissue to complete the circuit. Current (200 μA) was applied via the electrode until a complete thrombotic occlusion had occurred, verified by a zero flow velocity on the Doppler flow recording. Before occlusion became complete, a pattern of cyclic flow variations, manifest by gradual decreases in flow velocity followed by sudden abrupt increases, was evident on the Doppler recording, similar to the pattern observed by others12,13 and attributable to intermittent accumulation and dislodgment of platelet thrombi.

Induction of clot lysis. Thirty minutes after the thrombotic occlusion had become complete, t-PA (recombinant human t-PA provided by Genentech, Inc., South San Francisco, Calif.; specific activity equivalent to 8×1010 IU/mg) was infused intravenously at a dose of 5 mg/kg over 60 minutes with a pump (Syringe infusion pump 22, Harvard Apparatus, South Natick, Mass.). Ten percent of the total dose was infused as a bolus during the first 2 minutes. Animals were given heparin (200 units/kg) and aspirin (30 mg/kg) intravenously over 2 minutes beginning 3 minutes before the infusion of t-PA. Recanalization was reflected by a return of average flow velocity to >50% of baseline values. Flow velocity was monitored continuously, and blood samples were obtained serially after completion of the infusion of t-PA. Administration of aspirin, heparin, and t-PA; carotid artery ligation; or introduction of cathodal current does not elicit an increase in plasma PAI-1 activity in this preparation.10 Because the initial experiments in sham-operated controls showed that surgical intervention exerted only a minor effect on plasma PAI-1, subsequent experiments were performed without an interposed interval after surgery.

Biochemical Procedures

t-PA antigen was assayed by an ELISA (American Diagnostica, Greenwich, Conn.). PAI-1 activity in citrated plasma was assayed spectrophotometrically with a modification of the assay developed by Chmielewska and Wiman14 as described previously. One arbitrary unit (AU) was defined as the amount of PAI-1 that would inhibit 1 IU of t-PA in a 10-minute interval. Standard curves were obtained with serial dilutions of pooled rabbit plasma. Results were expressed as AU per milliliter.

Preparation of cDNA probes, isolation of RNA, and assay of PAI-1 mRNA by Northern blotting. Rabbits subjected to thrombosis and to thrombolysis were given an overdose of pentobarbital at the conclusion of the experiments. Organs were removed rapidly by prompt dissection, minced, rinsed twice in phosphate-buffered saline (PBS) at 0–4°C, frozen quickly by liquid nitrogen, and stored at −70°C. Total RNA was prepared from frozen tissues by the acid guanidium thiocyanate–phenol–chloroform method,15 and the concentration of RNA was measured spectrophotometrically (A260 nm). The integrity of the RNA isolated was verified by ethidium bromide staining of ribosomal RNA. A 0.9-kb PAI-1 complementary DNA (cDNA) probe was generated by digestion of PAI-1 cDNA provided by T.-C. Wu16 (Monsanto, St. Louis, Mo.) with EcoRI and Sal I. The probe was isolated by batch affinity adsorption with sodium iodide beads (Geneclone, Bio 101, La Jolla, Calif.). A probe for glyceraldehyde-3-phosphate dehydrogenase mRNA (GAP) was obtained by XbaI/HindIII digestion of cDNA (#57091, American Type Culture Collection, Rockville, Md.). Its integrity was verified by ethidium bromide staining after agarose gel electrophoresis. cDNA probes were labeled with deoxyctydine 5'-[d-32P]triphosphate (Amersham, Arlington Heights, Ill.) by the random primer technique (Boehringer Mannheim, Indianapolis, Ind.) to a specific activity of 1×109 dpm/μg.

Total RNA (5–20 μg) in each sample was separated by 1.5% formaldehyde agarose gel electrophoresis. Northern blotting was performed by capillary transfer to nylon membranes (GeneScreen, DuPont–New England Nuclear, Wilmington, Del.). Blots were exposed to 80°C for 2 hours in a vacuum, and hybridization was

| Table 1. Efficacy of t-PA in Inducing Recanalization After Carotid Artery Thrombosis in the Experimental Animal Preparation Used |
|-----------------------------|------------------|------------------|
| Recanalization | Time to initial occlusion (minutes) | Peak concentration of plasma t-PA antigen (μg/ml) |
| Not induced | 11 | 56.2±24.4 | 24.5±8.8 |
| Induced | 15 | 60.9±24.6 | 22.0±8.6 |

* t-PA, tissue-type plasminogen activator. Values are mean±SD.
performed conventionally as described previously. In brief, membranes were prehybridized in a solution of 50% deionized formamide, 10× Denhardt’s solution, 0.05 M Tris-HCl, 1.0 M NaCl, 0.1% sodium pyrophosphate, 1% sodium dodecyl sulfate (SDS), 10% dextan sulfate, and 100 μg/ml sonicated salmon sperm DNA for at least 6 hours at 42°C. Hybridization with 320,000 dpm/ml (PAI-1) and 160,000 dpm/ml (GAP) of labeled probes was performed at 42°C for 20–24 hours. Membranes were then washed three times for 20 minutes each at 55°C with 1% SDS and with 2×, 1×, and 0.5× stock sodium chloride/sodium citrate solution (SSC) (1×SSC=0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). Blots were assayed with an Ambis radioisotopic scanner (Automated Microbiology Systems, San Diego, Calif.) for quantification of radioactivity corresponding to each band.

Hybridization to the GAP probe was used for calibration as an internal control and to ensure the provision of a consistent quantity of nondegraded RNA samples on each blot after transfer and hybridization. Autoradiography was performed with XAR-5 film (Kodak, Rochester, N.Y.) and intensifying screens (Cronex Lightening Plus, Dupont–New England Nuclear) at −70°C. The size of detected mRNA species was calculated on the basis of extent of migration from the gel wells.

**In Situ Hybridization**

**Tissue processing.** In situ hybridization was performed as previously described. In animals that had been subjected to thrombosis and thrombolysis, anesthesia was maintained with isoflurane, and the left ventricle was perfused with 0.9% saline followed by 4% paraformaldehyde in PBS, pH 7.3. Tissues were dissected...
FIGURE 3. Bar graph shows average increase in plasminogen activator inhibitor type 1 (PAI-1) messenger RNA (mRNA) in selected tissues from animals with thrombosis in which recanalization was not induced with tissue-type plasminogen activator (t-PA) and from animals in which recanalization was induced. The extent of the increase in PAI-1 mRNA was determined by comparison with values in control tissues from sham-operated animals given t-PA. All tissues were harvested 4 hours after the onset of infusion of t-PA. The increases noted represent the average change compared with values in sham-operated control rabbits in four or more preparations.

rapidly, placed in 4% paraformaldehyde in PBS for 4 hours, and frozen at −70°C. Cross sections (6 µm) were prepared with a cryostat and placed onto 0.01% poly-L-lysine–coated glass slides and stored at −70°C with desiccant. The sections were pretreated with proteinase K (1 µg/ml, 37°C, 30 minutes) and acetic anhydride (0.25% vol/vol in 0.1 M triethanolamine, pH 8.0), washed with 2×SSC (2×2 minutes), dehydrated in graded alcohols, and vacuum dried for 1 hour. In situ hybridization was initiated by the addition of 35S-riboprobe (375,000 dpm) in 75 µl of hybridization buffer (50% formamide; 0.3 M NaCl; 20 mM Tris, pH 8.0; 5 mM EDTA; 1× Denhardt’s solution; 10% dextran sulfate; and 10 mM dithiothreitol [DTT]). After hybridization for 20–24 hours at 65°C, the sections were washed four times for 5 minutes each with 4×SSC, treated with ribonuclease (20 µg/ml) for 30 minutes at 37°C, washed with 2×SSC twice for 5 minutes each, once with 1×SSC for 10 minutes, once in 0.5×SSC for 10 minutes, and once in 0.1×SSC at 75°C for 30 minutes. The tissue was then washed in 0.1×SSC and dehydrated by immersion in graded alcohols containing 1 mM DTT. Sections were dried and coated with NTB2 nuclear emulsion (Kodak) and incubated in the dark at 4°C for 2 weeks. After development, the sections were stained with hematoxylin and eosin.

Preparation of probes. For use for in situ hybridization, a 900–base pair fragment of a full-length cDNA of PAI-1 was cloned into Bluescript vector (Stratagene, La Jolla, Calif.). After being linearized, the cDNA was transcribed with T7 polymerase (Stratagene) to generate an antisense cRNA that was labeled with uridine 5′-d-[35S]triphosphate (Amersham, 800 Ci/mmol). With T3 polymerase and the same construct, a sense complementary RNA (cRNA) was generated for use as a negative control.

Identification of cell types. To identify cell types expressing PAI-1, the autoradiographed slides were stained with hematoxylin and eosin and examined by light microscopy at high magnification. In some cases, serial sections were processed for immunocytochemistry with markers specific for endothelial cells, including goat anti-human factor VIII–related antigen IgG (Atlantic Antibodies, Stillwater, Minn.) and goat anti-rabbit thrombomodulin antiserum kindly provided by Dr. Naomi Esmon. Immunochemistry was performed with the Vectastain ABC Elite peroxidase system (Vector Laboratories, Burlingame, Calif.). The final reaction products were produced with a 3,3′-diaminobenzidine substrate kit that yields a brown color for positively stained material. It became apparent that endothelial cells that were positive for factor VIII–related antigen or thrombomodulin constituted a linear monolayer of cells with flat nuclei abutting on parenchymal cells in liver, heart, and adrenal glands.

Analysis of Slides

Slides were analyzed on a Nikon microscope (Nikon, Tokyo). Four separate sections from each tissue were subjected to in situ hybridization and immunocytochemistry and analyzed by two independent observers. For in situ hybridization studies, to directly compare signal intensities from multiple experiments, the following type of control was used. A sample section from a previous experiment and a section from a control animal were included each time a new experiment was to be analyzed. These sections were mounted on the same slide, side by side with the sample section to be analyzed, and all components were subjected to the same processes throughout preparation.

Statistical Analysis

Data are presented as mean values ±SD. Comparisons between groups were made with two-tailed Student’s t test for paired or unpaired observations as appropriate. Wilcoxon’s nonparametric test was used for analysis of data in which the distributions were
unequal. A value of $p \leq 0.05$ was considered to be significant.

Results

Effects of Thrombolysis on Plasma PAI-1

Persistent, complete thrombotic occlusion was induced in 26 rabbits, occurring in an average of 58.8±24.1 minutes after initiation of electrical stimulation. Infusion of t-PA failed to induce recanalization in 11. In 15 (58%), recanalization was induced in an average of 46.6±20.7 minutes. Peak t-PA antigen levels and the initial interval preceding occlusion were similar in these two groups (Table 1). Recanalization was compromised by partial recollection reflected by cyclic flow variations in five rabbits and by complete thrombotic occlusion in six. Compromise of recanalization was evident only after the infusion of t-PA had been completed and occurred in an average of 115.8±28.4 minutes after initial recanalization.

Plasma PAI-1 activity increased significantly from 6.5±2.3 AU/ml at baseline to 41.9±22.9 AU/ml ($p<0.0005$) 4 hours after the onset of infusion of t-PA in rabbits in which thrombi had been induced and occlusion had persisted despite administration of t-PA. These values were similar and not significantly different from those in rabbits that had not been given t-PA (PAI-1 increased from 7.4±2.9 AU/ml at baseline to 30.5±15.9 AU/ml 4.5 hours after occlusion, $n=3$, $p<0.01$, Figure 1). When infusion of t-PA did induce recanalization, however, a more marked increase occurred. Thus, PAI-1 increased from 6.1±1.9 to 128.9±47.8 AU/ml 4 hours after the onset of infusion of t-PA ($p<0.0005$). The maximal increase was significantly greater than that in rabbits in which recanalization did not occur ($p<0.001$ compared with results in rabbits with occlusion that had not been given t-PA and $p<0.0005$ in rabbits with persistent occlusion despite administration of t-PA) even though baseline values and values at the time of occlusion were similar in all three groups. PAI-1 values at the time of occlusion were modestly increased in all three groups (Figure 1). In sham-operated controls in which the flow probe, plastic tube, and electrode had been placed and aspirin, heparin, and t-PA had been given but no current was applied and no thrombosis was induced, PAI-1 did not increase significantly (5.3±1.5 AU/ml at baseline and 9.6±7.4 AU/ml 4 hours after the onset of infusion of t-PA, $n=3$).

Effects of Thrombosis and Thrombolysis on Expression of PAI-1 mRNA

To characterize expression of the PAI-1 gene in vivo, total RNA was extracted from tissues of rabbits subjected to thrombosis and thrombolysis, and 5 µg was analyzed for PAI-1 mRNA expression by Northern blotting. Results from representative experiments are shown in Figure 2. As previously noted, only one PAI-1 mRNA species (3.2-kb) was detectable, compared with human tissues, in which both a 3.2-kb and a 2.2-kb species are evident. In rabbits subjected to thrombosis, PAI-1 mRNA levels increased in aorta, heart, and liver (lanes 1–3) compared with results in sham-operated controls (lanes 7–9). PAI-1 mRNA levels in sham-operated controls did not differ detectably from those in normal rabbit tissues (n=3 rabbits in each group). In rabbits subjected to thrombolysis after thrombosis, the increases in PAI-1 mRNA were more marked (lanes 4–6). The increases in tissue PAI-1 mRNA occurred without any apparent changes in the concentration of GAP mRNA. More modest increases of PAI-1 mRNA were detectable in lung, kidney, and spleen when greater amounts of RNA (20 µg) were analyzed. No increase was seen in hind leg skeletal muscle PAI-1 mRNA. Taken together, these results indicate that increased PAI-1 mRNA expression occurs in multiple organs, but particularly in aorta, heart, and liver.

To delineate the average increase in PAI-1 mRNA in specific tissues, groups of animals were studied as indicated in Figure 3. The amount of PAI-1 mRNA in samples from tissues was determined by comparison with tissues from control rabbits. Increases in PAI-1 mRNA were expressed as multiples of values in tissues from controls. Although variations were noted between animals within each group, a pattern of gene expression consistent with that indicated by the results in Figure 2 was evident. A twofold to threefold increase was seen in animals subjected to thrombosis but in whom recanalization was not successfully induced. A fourfold to sixfold increase was seen in animals in which recanalization was induced by t-PA. The greatest induction occurred in aorta, followed by heart and liver. Increases of twofold or less were seen in some other tissues.

The percentages of animals in which PAI-1 mRNA increased in response to thrombosis and to thrombolysis are shown in Figure 4. Although the extent of induction varied from animal to animal, increases in PAI-1 mRNA were detected in all samples of aorta from animals that had exhibited recanalization. Tissues other than aorta, heart, and liver exhibited induction less frequently and more modestly.

Cellular Localization of PAI-1 mRNA

The identity of specific cells in tissue that produce PAI-1 mRNA was determined by in situ hybridization. Serial 6-µm sections of tissues from five rabbits (two control, two with recanalization induced by t-PA, and one in which recanalization had not been induced successfully) were exposed to 32P-labeled PAI-1–specific cRNA probes. Although most cells in control tissues did not hybridize with the PAI-1 mRNA probe, a weak but specific signal was demonstrable in endothelial cells of...
the heart, aorta, and liver. After thrombosis and thrombolysis, stronger signals were evident in these tissues, and some other tissues became positive. In general, a single population of cells was identified as the source of positive signals regardless of the tissue examined—namely, endothelial cells. This finding was consistently and reproducibly observed in sections from separate experiments. Markedly increased expression of PAI-1 mRNA was evident in endothelial cells in rabbit aorta obtained after thrombolysis (Figure 5). Augmentation of PAI-1 mRNA expression was much less prominent in tissues from animals subjected to thrombosis alone in which t-PA had failed to induce thrombolysis or in tissues from control animals. No specific hybridization signal was detectable when the sense (negative control) probe was used, verifying the specificity of hybridization.

Evaluation of serial sections in which endothelial cells were delineated by immunocytochemistry with antibody to factor VIII–related antigen or to thrombomodulin indicated that PAI-1 mRNA was present primarily in endothelial cells (Figure 5). Increased expression of PAI-1 mRNA was found also in the flat sinusoidal endothelial cells with dense nuclei in liver obtained after thrombolysis (Figure 6). In contrast, hepatocytes, which contain relatively pale, large, round, and centrally located nuclei with scattered clumps of chromatin, did not appear to express PAI-1 mRNA.

Significant expression of PAI-1 mRNA was seen in endothelial cells of vessels in samples of heart and adrenals (Figure 7). Additional signals were observed over interstitial cells in heart and adrenals, probably representing labeled capillary endothelial cells, but individual capillaries could not be identified unequivocally. Positive signals were evident also in endothelial cells of glomerular capillary tuft and peritubular vessels in kidney (Figure 7). No signal was detected over the epithelium of proximal or distal renal tubules or collecting ducts.

In lung, positive signals were found in endothelial cells of the pulmonary arteries and arterioles. In contrast, airway epithelial cells exhibited no signal. PAI-1 mRNA expression was much less prominent in the same tissues from animals subjected to thrombosis alone in which t-PA had failed to induce thrombolysis and from control animals. No specific hybridization signal was detectable when the sense (negative control) probe was used (data not shown).

These results indicate that the primary site of PAI-1 gene expression after thrombosis and thrombolysis in vivo is endothelium. In aorta, almost all endothelial cells appeared to express PAI-1 mRNA. In liver and heart, however, only some endothelial cells expressed PAI-1 mRNA. Others, identified by immunoreactivity to anti-factor VIII–related antigen, did not. Thus, specific subsets of endothelial cells may be responsible for the enhanced expression of PAI-1 mRNA under these conditions, even though the fractions of cells positive for factor VIII–related antigen were similar in tissues from control animals and animals subjected to thrombosis with or without recanalization.

Discussion

In previous studies, we have shown that platelet lysates increase steady-state PAI-1 mRNA levels in cultured human endothelial cells and increase plasma PAI-1 in rabbits in vivo. Furthermore, we showed that activation of platelets, manifested by cyclic flow variations, increases plasma PAI-1 in vivo. In the present study, we demonstrated that activation of platelets in vivo (secondary to arterial injury and to thrombolysis) increases PAI-1 expression in diverse tissues manifested by increased PAI-1 mRNA in vivo. Responses were greatest in aorta, heart, and liver. In situ hybridization demonstrated that the increase in PAI-1 mRNA was virtually specific to endothelial cells, consistent with the known PAI-1 biosynthetic capacity of endothelial cells in culture.

In clinical studies, elevations in plasma PAI-1 have been strongly associated with an increased risk of thrombosis. Unstable angina at rest is accompanied by elevated PAI-1 activity as well as by intracoronary thrombi. Decreases in PAI-1 activity have been adjudged to predispose to bleeding. In experimental animals, increased circulating PAI-1 is prothrombotic. Thus, elevations in circulating PAI-1 may suppress systemic fibrinolysis by inhibiting circulating plasminogen activators and suppress fibrinolysis locally in the vicinity of clots with consequent predisposition to thrombosis.

Our results suggest that the increases in plasma PAI-1 associated with thrombosis and thrombolysis reflect changes in expression of the PAI-1 gene that antecedent increases in plasma PAI-1 and appear to underlie them. Modulation of the increased PAI-1 gene expression may therefore potentiate the efficacy of treatment with thrombolytic agents or attenuate the risk of thrombosis in patients predisposed to vascular insults.

The specific factors responsible for increased plasma PAI-1 and augmented PAI-1 gene expression observed in vivo in this study have not yet been elucidated definitively. Although platelets do not appear to contribute a major source of plasma PAI-1 under physiological conditions, they contain substantial amounts of the protein. Thus, under pathophysiological conditions, release of PAI-1 from platelets upon activation may contribute to an early increase in PAI-1. This may account for the modest increase in PAI-1 seen at the time of occlusion in the present study.

Thrombolysis induced a particularly marked increase in plasma PAI-1. Several explanations may pertain. Platelet activation is known to occur when thrombi are
lysed in experimental animals and in human patients. Platelets may be activated by plasmin or plasmin-dependent induction of thrombin activity\(^2\)7 or by the result of their exposure to collagen in the vessel wall at sites of injury or to residual thrombi that are rich in procoagulant proteins including thrombin.\(^2\)8 Transformation of the PAI-1 molecule from a latent to an active form by exposure to matrix components may contribute. Phospholipids, major constituents of cell membranes, can activate latent PAI-1 in vitro.\(^2\)9 In our experiments, it is possible that occult pulmonary emboli, occurring as a result of the shunt procedure, may have induced further local platelet activation and PAI-1 release after recanalization.

In the circulating blood, PAI-1 has a relatively short half-life.\(^3\)0 Thus, steady-state concentrations are maintained by a relatively high rate of synthesis. Concentrations of PAI-1 increase rapidly in response to several agents and to changes in physiological state,\(^3\)1 suggesting that synthesis is highly regulated. The short half-life of circulating PAI-1 and the long interval between induction of thrombosis and the observed increase in PAI-1 suggest that the increase in PAI-1 is at least partly attributable to a delayed increase in endogenous synthesis analogous to that seen in our previous studies in vitro.\(^6\),\(^7\)\(^2\)0 Factors potentially mediating the augmented PAI-1 gene expression include platelet-associated growth factors\(^8\) and thrombin.\(^2\)8

PAI-1 mRNA is present in several highly vascularized human tissues including placenta, uterus, myocardium, and liver.\(^6\) In rats, PAI-1 mRNA is prominent in lung.\(^3\)2 In mice, it is prominent in aorta, lung, adipose tissue, and heart.\(^3\)3 The presence of low but detectable levels of PAI-1 mRNA in diverse tissues suggests that a cell type common to each may be pivotal. Our results demonstrate the importance of endothelium.

Several limitations in our study merit consideration and relate to 1) the possibility that thrombosis with another plasminogen activator might affect PAI-1 differently; 2) uncertainty regarding the mechanism implicated (platelet activation contributing to increased PAI-1 gene expression and synthesis); 3) lack of direct proof that the increased expression of the PAI-1 gene that we observed is paralleled by increased translation of PAI-1 protein and that the markedly increased plasma PAI-1 activity is attributable only to newly synthesized protein; and 4) variance in subpopulations of endothelial cells in terms of the response to the stimuli used and expression of PAI-1 mRNA. Nevertheless, it seems likely that the increased plasma PAI-1 we observed after thrombosis reflects, at least in part, synthesis by endothelial cells because of the delayed marked increase in plasma PAI-1 activity and the preceding increase in PAI-1 gene expression. The absence of an increase in PAI-1 mRNA in hepatocytes and epithelial cells suggests that neither parenchymal liver cells nor epithelial cells contributed significantly.

Our results indicate that PAI-1, a potent antifibrinolytic protein, is produced by endothelial cells in diverse organs and that its augmented expression after thrombosis or thrombolysis may contribute to the antifibrinolytic rebound seen after and potentially compromising pharmacologically induced thrombolysis.\(^3\)4,\(^9\) Accordingly, attenuation of the increased PAI-1 gene expression in endothelium in vivo is an attractive target for enhancing the therapeutic efficacy of coronary thrombolysis, although it may increase the risk of bleeding.\(^2\)4 In addition, our results suggest that occult thrombosis may be perpetuated and exacerbated by augmentation of expression of PAI-1 in the vessel wall and that the augmented expression may be a potent link between thrombosis and the pathogenesis of vascular disease dependent on altered tissue remodeling.

Acknowledgments

We thank Kenneth Schechtmans, PhD, for statistical support, Naomi Esmon, PhD, for providing antithrombomodulin antiserum, Denise Nachowiak, John Botz, Jeffrey Labuda, and Pamela Lundius for their technical skills, and Barbara Donnelly and Lori Dales for secretarial assistance.

References


Figure 7. Facing page. Color photomicrographs showing significant expression of plasminogen activator inhibitor type 1 messenger RNA in endothelial cells of larger vessels in the heart (panel A) and capillaries in the connective tissue spaces of adrenal gland (panel B). Glomerular and peritubular endothelial cells exhibited expression as well (panel C), as did arterial endothelial cells in kidney (panel D). The tissues depicted were from an animal with thrombosis in which recanalization was induced with tissue-type plasminogen activator and were obtained under the conditions described in the legends to Figures 2 and 3. Original magnification, \(\times750\).


34. Rapold HJ, Grimaudo V, Declerck PJ, Kruithof EKO, Bachmann F: Plasma levels of plasminogen activator inhibitor type 1, β-thromboglobulin, and fibrinopeptide A before, during, and after treatment of acute myocardial infarction with alteplase. Blood 1991;78:1490–1495
Induction of endothelial cell expression of the plasminogen activator inhibitor type 1 gene by thrombosis in vivo.
S Fujii, H Sawa, J E Saffitz, C L Lucore and B E Sobel

Circulation. 1992;86:2000-2010
doi: 10.1161/01.CIR.86.6.2000
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/86/6/2000

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 Circulation is online at:
http://circ.ahajournals.org//subscriptions/