reviews

The Role of Thrombin and Thrombin Inhibitors in Coronary Angioplasty*

Mir Nadir Ali, MD; G. Villarreal-Levy, MD; and Andrew I. Schafer, MD

(CHEST 1995; 108:1409-19)

ACT=activated coagulation time; aPTT=activated partial thromboplastin time; AT III=antithrombin III; ECM=extracellular matrix; F 1.2 = fragment 1.2; FPA=fibrinopeptide A; GAG=glycosaminoglycan; PC=protein C; AT III= inhibitor; TX=tissue factor; TF=tissue factor; Th=thrombin; Pl=platelet phospholipid membrane; PC=protein C; APC=activated protein C; TM=thrombomodulin.

Key words: angioplasty; antithrombin; heparin; hirudin

Percutaneous coronary angioplasty has become an established modality in the treatment of obstructive coronary artery disease. The dilatation of the stenosed coronary artery relieves luminal narrowing but inevitably results in plaque rupture and exposure of subendothelium. The latter two events are potent stimuli for activation of coagulation and deposition of platelet-fibrin thrombi. Despite the use of aspirin and intravenous heparin during the procedure, there is a 6 to 8% incidence of acute coronary occlusion, occurring either intraprocedurally or within 24 h after balloon dilation.1-3 Acute occlusion accounts for most of the initial mortality, emergency bypass surgery, and morbidity of the procedure.4 In addition, nonocclusive mural thrombus, which is invariably present after balloon injury of diseased coronary artery and is thought to function as a source of growth factors from platelets and a scaffold for the migration and proliferation of smooth muscle cells (SMCs), culminates in restenosis in 30 to 50% of patients undergoing the procedure.5-10

Thrombin plays a crucial role at several levels in the cascade of reactions leading to mural thrombus formation. In addition, it regulates many postthrombotic cellular events like chemotraction of inflammatory cells and the growth regulation of endothelial and vascular SMCs.11-13 Thus, thrombin is implicated in both acute coronary occlusion and late restenosis following coronary angioplasty.14

Elucidating the mechanisms of thrombin-mediated clotting and cellular events as well as its inhibition by antithrombin III-(AT III)-dependent and direct-acting thrombin inhibitors has been intensively investigated over the last decade. Accordingly, this review will focus on the role of thrombin in acute coronary occlusion and restenosis following angioplasty, as well as the potential role that thrombin inhibitors might play in preventing the adverse consequences of the procedure.

Role of Thrombin After Vessel Injury

The generation of thrombin at the site of balloon injury is thought to occur in the following manner. Coronary angioplasty results in endothelial denudation and tears of the media. This results in exposure of subendothelial extracellular matrix (ECM), which serves as a potent stimulus for activation of both the

Figure 1. The interaction of AT III and hirudin, a prototype direct thrombin inhibitor, with the coagulation cascade. The diagram demonstrates inactivation of several enzymes of the coagulation pathway by AT III in contrast to the inhibition of thrombin only with direct thrombin inhibitors. PL=platelet phospholipid membrane; TF=tissue factor; Ca=calcium; Th=thrombin; PC=protein C; APC=activated protein C; TM=thrombomodulin.
Fibrin

\[ \text{to} \]

Fibrinogen

\[ \text{activating} \]

clot-associated D).

Thrombin

\[ \text{interact} \]

with exosite

inhibitors, thrombin

zymatically

tear

generated by

exosite,

change

substrate.

AT III

is

inhibit thrombin

by,

AT III-heparin complexes (Fig 2), thus rendering it less sensitive to inhibition by heparin. It is thought that the binding of thrombin to clot or ECM produces an allosteric modification masking the heparin-binding site of the enzyme. The clot-associated thrombin requires a 20-fold higher concentration of heparin compared with soluble thrombin to achieve an equivalent degree of inhibition.

The time course of thrombin generation has been evaluated after balloon injury of the rabbit aorta. Thrombin activity peaked at 5 h at a rate of 50 fmol/min/cm² of injured vessel wall. It declined steadily to a rate of 10 fmol/min/cm² of vessel surface at 24 h and remained at this level for as long as 10 days. The rabbit model, however, does not duplicate coronary angioplasty in humans.

Balloon injury in this experimental model is performed on normal aorta and results in endothelial denudation without deep medial tears. Thus, the rate and extent of thrombin generation is likely to be higher and its time course more prolonged after angioplasty-induced deep arterial injury of diseased coronary arteries in humans.

The effects of thrombin on the intimal surface after vessel injury are not only mediated through its role in the coagulation cascade but also via its potent activation and aggregation of platelets. Platelets respond to a variety of agonists by activating their common pathway for aggregation via the interaction of the membrane glycoprotein IIb/IIIa complex with fibrin(ogen) and von Willebrand factor.

Of the different potential platelet agonists that induce aggregation, thrombin is the most potent. Thrombin promotes its procoagulant and growth regulatory effects on the injured vessel wall via the aggregation of platelets, which release growth factors and cytokines with potent mitogenic activity directed at intimal and medial SMCs.

Thrombin regulates its own generation by a feed-
back mechanism that depends on the nature of the vascular substrate to which it binds. When endothelium is intact, the surface glycoprotein thrombomodulin binds thrombin and changes its catalytic specificity toward activation of anticoagulant protein C rather than procoagulant fibrinogen. Activated protein C reduces thrombin generation by destroying factors Va and VIIIa, which are integral components of the tenase and prothrombinase complexes, respectively (Fig 1). Thus, an intact vascular endothelium quenches thrombin generation. However, with endothelial denudation, thrombin activity is directed at activation of factors V and VIII, amplifying the activity of the tenase and prothrombinase complexes and thereby increasing thrombin generation.

The fibrin- and ECM-bound thrombin can function as an SMC mitogen. This concept has gained further support by the identification of a cellular thrombin receptor, and by the recent demonstration of messenger RNA (mRNA) for this receptor in human atherosclerotic plaques. The binding of thrombin to its receptors elicits G protein-mediated protein kinase activation and the subsequent induction of platelet-derived growth factor A-chain gene expression in SMCs. In addition, both AT III and direct thrombin inhibitors like hirudin reduce SMC proliferation in vitro and neointimal hyperplasia and angiographic restenosis after balloon-mediated vascular injury in animal models.

**AT III and Heparin**

The major physiologic anticoagulant in blood that regulates the activity of thrombin and other activated clotting factors (serine proteases) in the coagulation cascade is AT III. The mechanism of protease inhibition by AT III was first described by Rosenberg. AT III forms a 1:1 stoichiometric complex with thrombin by interaction between the arginine reactive center of the inhibitor and the serine active center of the protease (Fig 3). Complex formation occurs at a very slow rate in the absence of heparin. However, the binding of heparin or other vessel wall glycosaminoglycans (GAGs) to the lysyl residues of AT III produces an allosteric modification in the position of the reactive arginine residue of the inhibitor and markedly accelerates its interaction with thrombin and other serine proteases.

Balloon-induced vessel injury accelerates the deposition of AT III, a serine protease inhibitor (serpin), on the subendothelium where it binds to the GAG, heparan sulfate. AT III binding peaks at 1 h and is sustained at this level for up to 7 days after vessel injury. Vessel wall GAGs are capable of catalyzing the thrombin-inhibitory activity of AT III by 2,000-fold, similar to exogenously administered heparin. Thus, AT III is present at the site of thrombin generation on

---

**Figure 3.** Interaction of AT III with thrombin and heparin. The binding of heparin to the lysyl residues of AT III produces an allosteric modification in the inhibitor and dramatically accelerates its interaction with thrombin. AT III forms a 1:1 stoichiometric complex with thrombin by interaction between the arginine-reactive site of the protease with the serine-active site of the serpin. The resultant covalent bond at the catalytic site of thrombin leads to its inactivation. Heparin dissociates from the complex and can bind to other AT III molecules.
the vessel wall and modulates its activity. The administration of exogenous heparin could be potentially thrombogenic for the injured vessel wall because plasma heparin has been shown to elute AT III from its GAG-binding sites.\textsuperscript{43,44} It is likely that heparin present in the circulation competes with heparan sulfate on the vessel wall for AT III binding, thereby reducing AT III bound to the injured vascular surface. Prolonged heparin infusions reduce circulating levels of AT III in plasma.\textsuperscript{45} The decrease in AT III levels with the use of periprocedural heparin has been described in experimental coronary angioplasty.\textsuperscript{33,46} In humans, a 25 to 27% reduction in AT III level at 24 h following coronary angioplasty has also been observed.\textsuperscript{47} This decrease in AT III levels with heparin use during angioplasty has the potential to enhance the thrombogenicity of the injured vessel surface. In vitro studies have suggested that clot-associated and extracellular matrix-bound thrombin is protected from inactivation by AT III-heparin complex.\textsuperscript{18,19} Nevertheless, AT III administration (bolus plus 1-h infusion) to rabbits after balloon-mediated denudation of the aorta produced marked suppression of thrombin activity at 3 h after vessel injury.\textsuperscript{41} The inhibition of thrombin activity by AT III administration was superior to that achieved with a 1- and 3-h infusion of heparin. In addition, supraphysiologic AT III levels in conjunction with heparin reduced angiographic restenosis when compared with heparin-treated controls in the oversized balloon injury model of coronary restenosis in swine.\textsuperscript{33}

The clinical observations that the efficacy of heparin is limited in angioplasty (abrupt vessel closure), unstable angina (ischemic events), and during thrombolysis for acute myocardial infarction (reocclusion) are consistent with the in vitro findings that fibrin-associated thrombin is protected from inhibition by the heparin-AT III complex. Fibrin(ogen) degradation products also bind to thrombin and mask the heparin-binding site, reducing the effectiveness of the anticoagulant during thrombolysis.\textsuperscript{48}

Heparin resistance may also be mediated by factors other than the inaccessibility of the anticoagulant-AT III complex to its thrombin binding site. The platelet-rich thrombus on the injured vessel surface releases platelet factor 4 (PF4) and other heparin-binding proteins that neutralize the anticoagulant activity of heparin.\textsuperscript{49,50} Extracts of PF4 from 1 mL of coronary thrombi of swine are capable of neutralizing 28 U of heparin or 140 volumes of therapeutically anticoagulated plasma (0.2 U of heparin per milliliter).\textsuperscript{49} Thus, the local antithrombin activity generated by the mural thrombus can potentially neutralize the anticoagulant effect of the agent.

**Hirudin and Related Peptides**

The limitations of heparin as an anticoagulant led to the development of direct-acting, AT III-independent thrombin inhibitors.\textsuperscript{51} The prototype among these is hirudin, a substance derived from medicinal leech, *hirudo medicinalis*.\textsuperscript{17,51} The recombinant forms of hirudin also possess potent antithrombotic properties despite the fact that they are not sulfated at tyrosine residue on position 63. Hirudin is a 65-amino acid polypeptide with molecular weight of 7,000 d or less. Hirudin inhibits thrombin by forming a 1:1 stoichiometric complex that is essentially irreversible, with a low dissociation constant of 20 fmol. When compared with the heparin-AT III complex, hirudin is more effective at inhibiting clot-bound thrombin in vitro.\textsuperscript{19}

Thrombin catalyzes the initial conversion of fibrinogen to fibrin and fibrinopeptide A (FPA), a small polypeptide with a short (3 to 5 min) half-life in plasma. FPA is a useful marker of thrombin activity (Fig 1). The ability of heparin, hirudin, and synthetic thrombin inhibitors to block FPA release by soluble and fibrin-associated thrombin was evaluated in citrated plasma (Fig 4-6). All thrombin inhibitors produced concentration-dependent inhibition of FPA release mediated by fluid-phase thrombin. Heparin, however, was much less effective against fibrin-bound thrombin, requiring a 20-fold higher concentration to block 70% of the clot-bound enzyme compared with equivalent inhibition of soluble thrombin (Fig 4). Hirudin was 50% as effective against clot-bound compared with fluid-phase thrombin, while smaller hirudin analogs and D-phenylalanyl-L-prolyl-L-arginyl chloromethyl ketone (PPACK), a synthetic inhibitor, were equipotent against the two forms of the serine protease (Fig 5, 6). It was
hypothesized that the smaller direct thrombin inhibitors are better able to penetrate into the interstices of the fibrin meshwork and thus achieve more potent inhibition of fibrin-bound thrombin (Table 1).

Hirudin binds to thrombin at the catalytic site and also at the anion binding exosite by an anionic hirudin tail fragment (Fig 2). The recognition of these binding characteristics led to the development of shorter peptide derivatives, like hirugen and hirulog. Hirugen is a synthetic tyrosine-sulfated dodecapeptide comprising residues 53 to 64 of the C-terminal region of hirudin.19 The antithrombin activity of hirugen is due to its interaction with the anion-binding exosite (Fig 2).17 The catalytic site of thrombin is not occupied by this inhibitor. The binding of this peptide to thrombin blocks fibrinogenolysis without affecting factor V activation or the amionic activity of the enzyme. Hirulog, a synthetic 20-amino acid peptide, was modeled to overcome the drawbacks of hirugen.50 Hence a tetrapeptide region, D-Phe-Pro-Arg-Pro, that interacts with the catalytic site of thrombin, was linked to the anion-binding tail segment of hirudin by a polyglycyl linker, achieving inhibition of both the fibrinogenolytic and amidolytic activities (Fig 2, 7). These shorter peptides derived from hirudin are equipotent inhibitors of clot-associated or soluble thrombin.19

**Table 1—Molecular Weights of Antithrombins**

<table>
<thead>
<tr>
<th>Antithrombins</th>
<th>Molecular Weight, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td>≥ average 15,000 (5,000-30,000)</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>58,000 (432 amino acid)</td>
</tr>
<tr>
<td>Hirudin</td>
<td>7,000 (65 amino acid)</td>
</tr>
<tr>
<td>Hirugen</td>
<td>524 (12 amino acid)</td>
</tr>
<tr>
<td>Hirulog</td>
<td>≈573 (20 amino acid)</td>
</tr>
<tr>
<td>Hirulog</td>
<td>1,578</td>
</tr>
</tbody>
</table>

**Thromboresistance (Passivation) of Vessel Wall Following Angioplasty**

The activation of coagulation factors, especially thrombin, with consequent platelet-fibrin deposition after balloon-mediated arterial injury produces a thrombogenic vessel surface. These thrombotic stimuli persist to a variable extent until endothelial regeneration can occur, which takes approximately 2 to 3 weeks. Even while the vascular intimal surface is reendothelialized, the thrombogenic stimuli are counterbalanced by physical factors (improved rheology following reduction of luminal narrowing) and by mediators that promote thromboresistance or passivation of vessel wall. The exact roles of rheology and the various mediators of vessel passivation, like AT III, tissue factor pathway inhibitor, protein C/thrombomodulin, tissue plasminogen activator, prostacyclin, and nitric oxide are uncertain. Without thromboresistance, there would be relentless progression toward vessel occlusion due to the unopposed activity of thrombin and ensuing platelet-fibrin thrombus formation. Only 6 to 8% of patients develop acute occlusion after coronary angioplasty, which supports the hypothesis that passivation may play a key role in maintaining vessel patency. However, there is paucity of data on the role of the potential biologic mediators of passivation, except for AT III and thrombin itself.53 The localization of AT III to the injured vascular surface is facilitated by endogenous sulfated GAGs on the subendothelium to which it binds with high affinity.20,41-43

**Figure 5.** Inhibition of fluid phase (cross-hatched lines) vs clot-associated thrombin (shaded) by hirudin. Alpha-thrombin (0.2 to 0.4 nmol) or fibrin clots were incubated with citrated plasma in the presence or absence of hirudin. At the end of the incubation period, the plasma level of FPA was measured by radioimmunoassay, and the percent inhibition of FPA generation was then calculated for each inhibitor concentration (reproduced with permission from Weitz et al).19

**Figure 6.** Inhibition of fluid phase vs clot-associated thrombin by hirugen. Alpha-thrombin (0.2 to 0.4 nmol) or fibrin clots were incubated with citrated plasma in the presence or absence of hirugen. At the end of the incubation period, the plasma level of FPA was measured by radioimmunoassay, and the percent inhibition of FPA generation was then calculated for each inhibitor concentration (reproduced with permission from Weitz et al).19
The addition of AT III to injured vessel wall has been shown to inhibit thrombin. In addition, administration of AT III prior to vessel injury in a rabbit aortic injury model reduces the amount of catalytically active thrombin on the vessel surface. These observations indicate that AT III is a mediator of vessel passivation after arterial injury.

Endothelial regeneration after vessel injury is a central event in arterial repair and may promote passivation. Experimental evidence indicates that thrombin facilitates reendothelialization through an arginine-glycine-aspartic acid (RGD) sequence that promotes endothelial cell adhesion. The thrombin bound to the fibrin mesh and GAGs at the site of injury can be modified by proteases derived from inflammatory cells to unmask an RGD binding site. This site interacts with the promiscuous vitronectin receptors on endothelial cell membranes with subsequent cell adhesion, actin microfilament assembly, and cell spreading on the injured vascular surface.

Thrombomodulin, which is constitutively present on endothelium, mediates an antithrombin effect by altering the specificity of thrombin from proteolysis of fibrinogen to protein C activation. Activated protein C reduces thrombin generation by inhibiting the formation of the tenase and prothrombinase complexes (Fig 1). However, balloon angioplasty causes endothelial denudation with consequent loss of a surface for thrombomodulin-protein C activation. These mediators, although important for thrombin inhibition in vessels with intact endothelium, are unlikely to be major contributors to vessel passivation after balloon arterial injury.

**Experimental and Clinical Studies With Heparin in Angioplasty**

Experimental and clinical studies concur on the importance of periprocedural anticoagulation with heparin for coronary angioplasty to reduce the incidence of acute occlusion. The importance of adequate heparin dosage on platelet deposition in the porcine angioplasty model was illustrated by Heras et al. There was an inverse relation between the number of labeled platelets deposited with the dose of heparin administered. These observations were further extended when recombinant hirudin was compared with heparin and platelet-fibrin deposition was evaluated after angioplasty. The direct thrombin inhibitor was superior to heparin in reducing the deposition of both fibrinogen and platelets, and fibrinogen in both the carotid and coronary arteries of swine after deep arterial injury.

The degree of anticoagulation achieved with heparin may be an important determinant of acute coronary occlusion after coronary angioplasty. In a study of 336 patients following elective percutaneous transluminal coronary angioplasty, myocardial infarction and coronary artery bypass surgery were more frequent in the group with activated partial thromboplastin time (aPTT) less than 3 x control, 6 to 18 h after angioplasty. Similarly, when activated coagulation times were evaluated in 1,469 consecutive patients, the group with in-hospital complications had lower activated coagulation times (ACTs) than patients without complications. These two retrospective studies do not address whether the lower ACT and aPTT in patients with complications was a cause or result of the adverse event. Acute myocardial infarction and ischemic coronary syndromes increase the synthesis of heparin-binding proteins and the local release of PF4 by platelets. This leads to heparin resistance and lower ACT and aPTT measurements. It is therefore possible that the groups with complications were significantly less anticoagulated because of the adverse events rather than suboptimal heparin dosage causing the adverse events.

There is general consensus that optimal anticoagulation with heparin appears to be necessary for prevention of acute complications of percutaneous transluminal coronary angioplasty. However, the role of heparin for prevention of late restenosis is less well defined. Experimental animal studies using the rat carotid artery model show a reduction in intimal hyperplasia (IH) with heparin-administration after balloon-mediated endothelial denudation. Thymidine incorporation studies revealed that the inhibition of IH was associated with reduction in SMC proliferation in the heparin-treated animals. There was no difference in the volume of ECM between the treated and control groups, indicating that the reduction of ECM...
neointima was the result of inhibition of SMC proliferation. The beneficial effects of heparin observed in laboratory animals did not translate into clinical benefit in humans. An 18- to 24-h infusion of heparin after angioplasty compared with procedural heparin only for prevention of restenosis showed no benefit, although the rate of angiographic follow-up was less than 60%.62 Bleeding complications, however, were twice as common in the heparin infusion group. A second trial that evaluated subcutaneous administration of heparin, 10,000 U daily, after coronary angioplasty showed a paradoxical increase in the incidence of restenosis (82% in the heparin group compared with 33% in the control group) and led to early termination of the study.65 The utility of heparin for reduction of restenosis, however, is not yet resolved. The results of these two clinical trials have prompted investigation of various dosages and routes of administration of heparin in the rat carotid artery model in an attempt to clarify the discrepancy between experimental benefit and clinical futility.54 The use of intermittent subcutaneous heparin was associated with a paradoxical increase in IH, while continuous intravenous infusion with osmotic pumps over a period of 14 days resulted in marked inhibition of neointima formation after balloon injury. Thus the benefit of heparin after angioplasty is uncertain since clinical studies have investigated its use over only a short period of 24 h or less.

EXPERIMENTAL AND CLINICAL STUDIES WITH HIRUDIN AND HIRULOG IN ANGIOPLASTY

Despite the periprocedural use of intravenous heparin, there is a 6 to 8% incidence of acute coronary occlusion, either intraprocedurally or within 24 h after vessel dilatation. More than half of the acute closures are due to coronary artery thrombosis. The failure of heparin in preventing this infrequent but potentially disastrous complication of angioplasty has led to the development of direct thrombin inhibitors. The use of these agents during angioplasty is appealing not only to prevent acute thrombotic occlusions (abrupt vessel closure) but also to potentially reduce the incidence of restenosis. In vitro experiments indicate that ECM- and fibrin-bound thrombin at the site of vessel injury are not only catalytically active but resistant to inhibition by heparin-AT III complexes.18,19 In contrast to heparin, direct thrombin inhibitors, depending on their molecular weight, are potent inhibitors of clot-associated or surface-bound thrombin (Table 1).19 While hirudin is only 50% as effective against clot-associated thrombin as against fluid-phase thrombin, hirugen is equally potent against both. These in vitro observations led investigators to evaluate the efficacy of these agents in animal models of platelet-fibrin deposition and restenosis after angioplasty.18,35,36,46,58 Hirudin had a potent antithrombotic profile, reducing platelet and fibrin deposition more effectively than heparin.58 This effect was observed especially in vessels with deep medial tears. Macroscopic mural thrombus was present in 57% of vessels treated with 50 U/kg heparin compared with 9% incidence in vessels treated with 1 mg/kg hirudin. These observations were extended to the coronary circulation by Buchwald et al.46 They evaluated the deposition of 111In platelets and 125I fibrinogen in miniature swine after implantation of balloon-expandable tantalum stents in coronary arteries. The animals were divided into three groups consisting of heparin bolus only, heparin bolus plus infusion, and recombinant hirudin, 1 mg/kg, bolus followed by infusion at a rate of 1 mg/kg/h. Platelet and fibrin deposition per stent was lower in the hirudin group compared with the two heparin groups.

Although acute thrombotic occlusion following balloon or stent injury is relatively infrequent, nonocclusive thrombus formation almost always occurs after angioplasty.57,58,61 Experimental studies indicate that such thrombus not only functions as a source of growth factors, but also provides a scaffold for subsequent migration and proliferation of SMCs.10 In addition, the catalytically active thrombin on the vessel wall regulates the growth of endothelial and vascular SMCs.12 Thus, the hypothesis that even brief periods of inhibition of the enzyme by direct thrombin inhibitors can reduce neointimal growth and restenosis was explored in two animal models. In atherosclerotic femoral arteries of rabbits, Sarembock et al.38 showed that recombinant hirudin bolus (1 mg/kg) followed by a 2-h infusion at 0.5 mg/kg/h reduced both angiographic (minimum lumen diameter) and morphometric (percent area stenosis) measures of restenosis at 4 weeks.35 Preliminary confirmation of these findings in the coronary arteries of miniature swine has also been reported.36 Only a few clinical studies involving the use of hirudin and related peptides have been reported. The use of hirudin in 74 patients with stable angina was prospectively compared with 39 matched heparin-treated patients.66 The dose of hirudin utilized, 20-mg bolus and 0.16-mg/kg infusion for 24 h, was relatively low compared with the doses used in animal experiments. Similarly, the dose of heparin used (10,000-U bolus and 12-U/kg infusion) was also lower than currently practiced.50,63 The hirudin-treated patients had less ischemia (ST segment changes) and clinical events (periprocedural myocardial infarction and need for emergency bypass surgery) than the heparin-treated controls. There was only a minor increase in bleeding from access sites in the hirudin-treated group (4/74 patients). APTT was more often in the target range in the hirudin group (50%) compared with the heparin group (3%) in the 24-h period of infusion.
A multicenter dose escalation study of hirulog in coronary angioplasty was performed to determine the efficacy and safety of this agent. Of the 291 patients, 151 in the three lower-dose groups were treated with 0.35 mg/kg or less bolus and 1.4 mg/kg/h infusion for 24 h. The median ACT in these three groups was lower than 300 s and the rate of abrupt vessel closure was high (10.2%). The remaining 128 patients in the two high-dose groups were treated with a hirulog dose of 0.45 mg/kg or more bolus and 1.8 mg/kg/h infusion for 24 h. The median ACT in these two groups was higher than 300 s and the incidence of abrupt vessel closure was 3.3%, similar to that reported in recent angioplasty literature.2 Spontaneous bleeding was low, with no patient experiencing severe life-threatening or intracranial bleeding.

These results with direct thrombin inhibitors in angioplasty are encouraging. However, they emphasize the need for phase 3 trials with these agents using appropriate doses needed for potent thrombin inhibition without compromising safety. The doses used in experimental studies are twofold to threefold higher for hirudin than utilized in the phase 2 study of patients undergoing coronary angioplasty. It appears unlikely that doses higher than this will be utilized following reports in three major trials of an excess of bleeding events in patients with acute myocardial infarction treated with hirudin and thrombolytic agents. The increase in intracranial bleeding was found only in patients given both thrombolytic agents and hirudin. With the use of hirudin alone for patients with unstable angina in the GUSTO 2A trial, no increase in bleeding events was observed. Similarly, since thrombolysis is rarely utilized during routine coronary angioplasty, higher doses of direct thrombin inhibitors can perhaps be used safely in this setting. Thus, trials addressing the use of hirudin for coronary angioplasty should resist the impulse to lower the hirudin dose as this may reduce the efficacy of this promising agent.

### Rebound Increase in Thrombin Activity With Cessation of Antithrombin Therapy

A major concern with the use of antithrombins for angioplasty is the so-called rebound phenomenon that refers to a resurgence of thrombin activity after cessation of heparin or direct thrombin inhibitors.66,72-74 Thrombin is a potent activator of factors VIII and V that are integral components of the tenase and prothrombinase complexes, respectively. The heparin-AT III complex decreases thrombin generation via two different mechanisms: (1) inhibition of several precursor enzymes of the coagulation cascade (factors Xa, XIIa, XIa, and IXa) that generate the tenase and prothrombinase complexes, and (2) via inhibition of thrombin, which in turn reduces the positive feedback activation of factors VIII and V.37

Hirudin and hirulog, in contrast, have no activity directed at the precursor enzymes in the coagulation pathway.17,75,76 Rather inhibition of thrombin generation is based on reduced factor VIII and V activation and consequent inhibition of positive feedback on the tenase and prothrombinase complexes.17,52,76 A recent phase 1 trial, however, indicates that hirudin at anticoagulant doses (20- to 10,000-fold molar excess over thrombin) does not block in vivo thrombin generation.77 Hirudin (0.1 to 0.3 mg/kg/h) in this study was infused for 6 h. There was a significant increase in markers of thrombin generation such as thrombin-hirudin complex and F1.2, both during and after cessation of hirudin infusion.

On cessation of infusion of antithrombins, a rebound increase in thrombin activity can possibly occur due to the unopposed activity of the prothrombinase complex on its substrate prothrombin. Rebound increase in thrombin activity on termination of infusion of argatroban, a direct thrombin inhibitor, for unstable angina has been reported.66,72 In this study, there was a correlation between biochemical manifestations of thrombin rebound (increase in FPA and thrombin-antithrombin complexes) and the recurrence of anginal episodes. In a phase 2 trial utilizing hirudin for angioplasty, no clinical evidence of thrombin rebound was noticed. However, during infusion of the drug, elevation in prothrombin F1.2 and thrombin-antithrombin complexes was observed. This would imply ongoing activity of the prothrombinase complex, with conversion of prothrombin to thrombin and F1.2. Similarly, the persistent generation of F1.2 in patients treated with hirudin for unstable angina suggests ongoing thrombin activation.74

The cessation of heparin infusion has also been associated with biochemical evidence of rebound thrombin activity following angioplasty. Manolis et al78 evaluated FPA activity as a measure of thrombin generation following angioplasty in 30 patients. They observed a fivefold increase in FPA levels over preangioplasty baseline approximately 4 h after cessation of heparin therapy. No major in-hospital complications were observed, possibly reflecting the small number of patients in the study. An increase in FPA and F1.2 within 3 h after completion of heparin infusion in patients with unstable angina or acute myocardial infarction has been reported in preliminary form.73 This biochemical evidence of rebound was variably associated with recurrent angina.

### Conclusions

In addition to its central role in hemostasis, thrombin, a multifunctional enzyme, mediates several nonhemostatic effects after vessel injury, including the following: (1) SMC proliferation; (2) chemotaxis of inflammatory cells; (3) degradation of ECM; and (4)
regulation of reendothelialization. These hemostatic and cellular effects of the serine protease contribute to acute vessel closure and late restenosis after angioplasty. Thrombin generation on the vessel wall at the site of injury peaks within 5 h and persists for approximately 10 days. Furthermore, thrombin generated on the vessel wall is either adsorbed onto the fibrin meshwork or the GAG in the subendothelium and is therefore protected from inactivation by heparin-AT III complexes. The sequestered enzyme may thus exhibit a prolonged, localized stimulation of the vessel wall milieu contributing to thrombosis and restenosis.

Heparin has significant limitations as an antithrombin for coronary angioplasty. First, its anticoagulant activity is mediated through AT III. Second, the thrombin present after vessel injury is bound either to fibrin or ECM and is resistant to inhibition by heparin. Third, heparin can increase the thrombogenicity of the vessel wall because it reduces circulating levels of AT III and can potentially dissociate AT III from the injured vessel surface. The administration of AT III along with heparin prior to angioplasty can perhaps overcome these limitations, as suggested by animal studies. However, the potential increased risk of bleeding with combination therapy (AT III plus heparin) needs careful preclinical review.

Hirudin and related peptides have the advantage of inactivating clot-associated thrombin, and consequently reducing stimuli for thrombus formation and cellular effects of thrombin at the site of angioplasty. Animal experiments have shown dramatic reduction in platelet-fibrin deposition after coronary angioplasty with hirudin. However, the preceding enzymes of the coagulation cascade are not inhibited by these agents and continued generation of thrombin during infusion of hirudin has been shown to occur. Also, potent inhibition of clot-associated thrombin may increase the risk of bleeding. It is likely that the systemic dose of thrombin inhibitors required to achieve adequate suppression of the serine protease may be associated with an unacceptable bleeding risk. To reduce the systemic effects, it may be necessary to develop innovative methods of local antithrombin therapy at the site of arterial injury. This would be feasible by improvement in in vivo percutaneous arterial gene transfer methods that direct the local expression of recombinant AT III or hirudin-like peptides. Alternatively, site-directed antithrombin effect would have to await development of noninflammatory and thromboresistant local drug delivery devices like eluting and/or biodegradable stents.

ACKNOWLEDGMENTS: The authors wish to thank Douglas L. Mann, MD, FCCP, and Judith Mickelson, MD, for their helpful review of the manuscript.

REFERENCES
14 Wilcox JN. Thrombin and other potential mechanisms underlying restenosis. Circulation 1991; 84:342-35
20 Hatton MW, Moor SL, Richardson M. Deendothelialization in vivo initiates a thrombogenic reaction at the rabbit aorta surface: correlation of uptake of fibrinogen and antithrombin III with thrombin generation by the exposed subendothelium. Am J Pathol 1980; 135:499-508
22 Takamatsu J, Horne MD, Granick HR. Identification of the thrombin receptor on human platelets by chemical crosslinking.


Esmen CT, Esmen NL, Harris KW. Complex formation between thrombin and thrombomodulin inhibits both thrombin-catalyzed fibrin formation and factor V activation. J Biol Chem 1982; 257:7944-47


Unterberg C, Buchwald A. Hirudin reduces neointimal proliferation after coronary angioplasty. Circulation 1994; 90:238A


Rosenberg RD. Biochemistry of heparin antithrombin interactions, and the physiological role of this natural anticoagulant. Am J CardioI 1989; 57:2S-3S


Hatton MW, Moor SL, Richardson M. Evidence that rabbit 125I-antithrombin III binds to proteoheparan sulphate at the subendothelium of the rabbit aorta in vitro. Blood Vessels 1988; 25:12-27


Edelman E, Karnovsky MJ. Contrasting effects of the intermittent and continuous administration of heparin in experimental
restenosis. Circulation 1994; 89:770-76


69 Sobel BE. Intracranial bleeding, fibrinolysis, and anticoagulation. Circulation 1994; 90:2147-52


