Selective Inhibition of Factor Xa Is More Efficient Than Factor VIIa–Tissue Factor Complex Blockade at Facilitating Coronary Thrombolysis in the Canine Model

JEFFREY LEFKOVITS, MBBS, JANIS L. MALYCKY, BS, J. SUNIL RAO, PhD,
CHARLES E. HART, PhD,* EDWARD F. PLOW, PhD, ERIC J. TOPOL, MD, FACC,
FRANCESCA A. NICOLINI, MD, PhD, FACC

Cleveland, Ohio and Seattle, Washington

Objectives. We determined the effect of adjunctive inhibition of the extrinsic coagulation pathway by factor VIIa–tissue factor complex inhibitors, DEGR VIIa and tissue factor pathway inhibitor (TFPI), and the selective factor Xa inhibitor, tick anticoagulant peptide (TAP), after thrombolytic therapy with tissue-type plasminogen activator (t-PA) in a canine model of electrically induced coronary thrombosis.

Background. Ongoing thrombin generation is considered an important component of the heightened thrombin activity associated with thrombolytic therapy and may be responsible for reperfusion failure and reocclusion.

Methods. Forty-two dogs with electrically induced coronary thrombus undergoing thrombolysis with t-PA (1 mg/kg over 20 min) were randomly assigned to one of the following adjunctive regimens: TAP (30 μg/kg body weight per min for 90 min, n = 10); TFPI (100 to 150 μg/kg per min for 90 min, n = 10); DEGR VIIa (1- to 2-mg/kg bolus, n = 10) and saline control (n = 12). The dogs were observed for 120 min after thrombolysis for reocclusion.

Results. All three active study agents accelerated the time to reperfusion by an average of 12 min (all p < 0.05). Duration of reflow was greatest with TAP (117 ± 8 min, p < 0.05 compared with saline control), whereas DEGR VIIa and TFPI did not prolong the duration of reflow. Reocclusion rates were similar among control, DEGR VIIa and TFPI groups (70%, 78% and 67%, respectively). Tick anticoagulant peptide reduced the occurrence of reocclusion (0%, p < 0.05 compared with saline control).

Conclusions. In this experimental model, during systematic blockade of various extrinsic coagulation pathway proteins, we demonstrated that whereas acceleration of thrombolysis occurs with factor VIIa–tissue factor complex inhibition, optimal enhancement of thrombolysis was achieved through specific factor Xa blockade.

(J Am Coll Cardiol 1996;28:1858–65)
©*1996 by the American College of Cardiology*

Despite substantial advances in the application of intravenous thrombolytic agents and the adjunctive use of antiplatelet and antithrombotic therapies, current thrombolytic intervention still does not achieve the goal of early, complete and sustained reperfusion in a majority of patients (1). Ongoing thrombin activity during thrombolysis is considered a major impediment to "optimal" reperfusion. Yet we and other investigators have found that agents such as heparin and hirudin, which inhibit thrombin activity but allow ongoing thrombin formation, may

not enhance thrombolysis as effectively as agents that specifically inhibit thrombin generation (2–4).

The extrinsic pathway is considered primarily responsible for intravascular thrombus formation in vivo (5,6). The exposure of subendothelial tissue factor (TF) during plaque fissuring and rupture results in the formation of activated factor VII–TF complexes and cascading activation of coagulation proteins, leading to generation of thrombin and fibrin formation. Recombinant DNA technology has recently made agents available that selectively inhibit key enzymes in the extrinsic coagulation pathway. Tick anticoagulant peptide (TAP) is a 60 amino acid protein derived from the soft tick, and is a potent and highly selective inhibitor of factor Xa (7). Recombinant tissue factor pathway inhibitor (TFPI) is a multivalent proteinase inhibitor whose complex actions result in inhibition of the factor VIIa–TF complex (8,9). Inhibition of the factor VIIa–TF complex can also be achieved by DEGR VIIa (ZymoGenetics)—an inactivated form of factor VIIa that still binds to TF but cannot catalyze factor X activation.

We hypothesized that adjunctive inhibition of the extrinsic coagulation pathway would prevent ongoing thrombin gener-

Abbreviations and Acronyms	
aPTT	= activated partial thromboplastin time
BT	= bleeding time
DEGR	= active-site inactivated factor VIIa
FPA	= fibrinopeptide A
PT	= prothrombin time
TAP	= tick anticoagulant peptide
TAT	= thrombin–antithrombin complex
TF	= tissue factor
TFPI	= tissue factor pathway inhibitor
t-PA	= tissue-type plasminogen activator

ation and enhance the efficiency of tissue-type plasminogen activator (t-PA)-induced thrombolysis. Adjunctive inhibition of the factor VIIa–TF complex by TFPI and DEGR VIIa was compared with selective factor Xa inhibition by TAP after thrombolytic therapy with t-PA in a canine model of electrically induced coronary thrombosis. The length of time required to achieve thrombolysis, extent of thrombolysis, incidence of acute rethrombosis and effects on markers of coagulation were evaluated.

Methods

Coronary artery thrombus formation. All procedures performed conformed to the guidelines of the American Physiological Society and were approved by the Animal Research Committee of the Cleveland Clinic Foundation. The methodology used to induce an occlusive coronary thrombus has been described in detail elsewhere (10,11). Briefly, a 2-cm length of the left circumflex artery was isolated in 42 anesthetized mongrel dogs of either sex, weighing 21 ± 2.0 kg. An ultrasonic Doppler flow probe (Crystal Biotech) was placed on the artery for coronary blood flow measurement. Thrombosis was induced using the electrolytic injury technique (12). The endothelium was manually injured; a silver-coated copper electrode wire with a 26-gauge needle tip was inserted into the isolated artery segment; and a vascular occluder, placed around the vessel distal to the flow probe and the electrode, was adjusted to induce ~80% diameter stenosis. Thrombosis was induced by the delivery of 100 μA of anodal current to the tip of the electrode, and the occluder was gradually removed after thrombus formation. Continuous mean aortic blood pressure and electrocardiographic monitoring was performed. Hemodynamic measurements were continuously recorded on a multichannel recorder (MFE). Catheters were inserted in both femoral veins and advanced into the inferior vena cava for infusion of the various therapeutic regimens and collection of blood samples.

Administration and dose selection of study agents. A period of 30 min was allowed to elapse after the formation of a fully occlusive coronary thrombus to confirm the stability of thrombus. The dogs then received t-PA (1 mg/kg body weight over 20 min) together with one of the following adjunctive treatments: 1) a control group of 12 dogs was given normal saline at a rate of 0.6 ml/min for 90 min; 2) 10 dogs received TAP at 30 μg/kg per min for 90 min; 3) recombinant TFPI was given at the initial dose of 100 μg/kg per min in 6 dogs and 150 μg/kg per min to 3 dogs, all for 90 min; and 4) DEGR VIIa was given to 7 dogs in a bolus of 1 mg/kg, and to 3 dogs in a bolus of 2 mg/kg.

The dose of TAP was based on previous experiments in our laboratory, which identified the threshold dose that suppressed increases in markers of thrombin activity, fibrinopeptide A and thrombin–antithrombin complexes during thrombolysis in dogs (4). We previously demonstrated effective inhibition of factor VIIa by DEGR VIIa in a cell-surface chromogenic assay using rat smooth muscle cells (13). Activity of DEGR VIIa against canine thromboplastin has also been demonstrated in in vitro clotting assays, although higher concentrations of DEGR VIIa were required with rabbit and baboon thromboplastin (unpublished data). The eventual dose of 1.0 mg/kg of DEGR VIIa was chosen based on the results of a dose-response study in the baboon, which found this dose to be in gross excess for the prevention of thrombus formation (14). The initial dose of TFPI was based on earlier studies demonstrating that doses between 50 and 100 μg/kg per min were associated with plasma levels of 2 to 5 μg/ml and inhibition of reocclusion after thrombolysis in a canine femoral artery model (15,16).

Dose regimens for each of the active study agents were reviewed at an interim analysis of the study results, which was prespecified in the study design protocol. If the incidence of reocclusion was ≥50% after the first six preparations, the dose of the study agent was increased. At the interim analysis, reocclusion had occurred in four of six dogs in the DEGR VIIa group and in three of six dogs in the TFPI group. The doses of TFPI and DEGR VIIa were therefore increased (by 50% for TFPI and by 100% for DEGR VIIa) for the remaining four dogs in the TFPI group and in three of the remaining four dogs in the DEGR VIIa group (the fourth dog did not receive the higher dose because of limited amounts of the study drug). There were no instances of reocclusion in the first six dogs in the TAP-treated group, and the dose of TAP was not changed.

Thrombolysis was defined as restoration of coronary blood flow to at least 30% of the baseline value, occurring at any time after the onset of t-PA infusion. Reocclusion was defined as occurrence of zero blood flow after successful initial thrombolysis. Dogs were observed for a 2-h period from the occurrence of thrombolysis for evidence of coronary reocclusion. In those dogs with intermittent restoration of coronary blood flow due to cyclic flow variations, the total duration of reperfusion was calculated as the length of time blood flow was greater than zero during the 2-h period after the onset of reperfusion.

Study agents. Recombinant t-PA was supplied as a powder in 50-mg vials by Genentech. DEGR VIIa was provided by ZymoGenetics, Inc. and was prepared as described previously (13). Recombinant TFPI was supplied by Monsanto Co. The preparation of this agent has also been previously described (16,17). Tick anticoagulant peptide was supplied by Merck & Co. and was prepared as previously described (4,18).

Table 1. Measures of Efficiency of Thrombolysis With Study Agents

	Saline Control (n = 12)	DEGR VIIa (n = 10)	TFPI (n = 10)	TAP (n = 10)
Reperfusion achieved	9 (75%)	9 (90%)	9 (90%)	10 (100%)
Time to reflow (min)	32 ± 13	$20 \pm 7^*$	$21 \pm 6^*$	$18 \pm 10^*$
Reflow duration (min)	62 ± 45	70 ± 48	91 ± 35	$117 \pm 8^*$
Cyclic flow variations (in dogs with initial reperfusion)	8 (89%)	7 (78%)	5 (56%)	0*
Reocclusion (in dogs with initial reperfusion)	8 (89%)	7 (78%)	6 (67%)	0*

*p = 0.05 versus saline control group. Data presented are mean value $\pm$ SD or number (%) of dogs. TAP = tick anticoagulant peptide; TFPI = tissue factor pathway inhibitor.

Coagulation activity studies and template bleeding time. Anticoagulated whole blood samples for prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin–antithrombin complex (TAT) and fibrinopeptide A (FPA) levels were collected at baseline (before thrombus formation), at the end of the t-PA infusion, at the end of the adjunctive therapy administration and at the end of the experiment (i.e., 1 h after the end of adjunctive treatment). Fibrinopeptide A concentrations were measured in plasma samples after fibrinogen was removed by Bentonite treatment by a modified procedure based on competitive enzyme-linked immunosorbent assay (ELISA) as previously described (4,19). Thrombin-antithrombin complex levels were determined using a commercially available ELISA kit (Asserachrom A.T.M., American Bioproduct Co.). Species cross-reactivity for both FPA and TAT was tested, with canine samples demonstrating 37% cross-reactivity for FPA and 100% cross-reactivity for TAT with human plasma samples (4,19). For gum bleeding time (BT) determination, an inner lip incision was made with a fully automated incision-making instrument (Surgicutt, International Technidyne Corporation). Blood flow was blotted every 30 s after the incision using blotting paper until bleeding stopped.

Measurement of TFPI and DEGR VIIa plasma levels. *Immunoassay of TFPI.* Plasma TFPI was measured by particle concentration fluorescence immunoassay as previously described (20). Standards and plasma samples were diluted in a buffer containing phosphate buffered saline, 10 mg/ml bovine serum albumin and 0.05% Tween 20, pH 7.2. A 40-μl aliquot was incubated at room temperature for 1 h with 15 μl of a 0.25% suspension of monoclonal anti-TFPI–IgG-bound carboxylpolystyrene particles. Anti-human TFPI antibodies labeled with fluorescein isothiocyanate were added (30 μl) and incubated for 30 min. The concentration of TFPI was measured by standard and sample comparative fluorescence concentration on a Pandex PCFIA analyzer (21).

Analysis of DEGR VIIa antigen. The plasma concentration of DEGR VIIa–treated dogs were determined using ELISA, as previously described (13). The assay uses a monoclonal-polyclonal sandwich format with an anti-human factor VIIa monoclonal antibody and a rabbit anti-human polyclonal antibody (both obtained from Walt Kisel, University of New Mexico). The concentration of DEGR VIIa in the dog plasma was determined by comparing the absorbance in the test sample to the DEGR VIIa standard curve.

Data analysis. Measures of thrombolytic efficacy for the four study agents were compared by analysis of variance (ANOVA). Special attention was paid to the multiple comparisons nature of this analysis. Dunnett's test was used to compare each of the three active agents' responses with the saline control. For pairwise comparisons among active treatment groups, a Bonferroni type correction was used to maintain the overall significance level at alpha 0.05. For categorical variables, pairwise comparisons were made using the Fisher exact test and Bonferroni type corrections. Three comparisons were performed in each of the contrasts, resulting in a critical value of p < 0.017, which was considered significant.

A repeated measures type of analysis was performed to determine treatment effects on coagulation variables and gum BT. Each dog's response curve over time was fitted into a summary slope measure for each of the variables measured. Treatment effect was determined by comparing the summary slope measures with ANOVA techniques. Because of the nonlinear nature of the response curves, summary slope measures included coefficients for both linear and quadratic effects. Multivariate ANOVA (Hotelling's T^2 test) was used to compare treatment effects. Because of the multiple (three) comparisons performed, a Bonferroni type correction was applied to maintain an overall significant of alpha 0.05, giving a critical value of p < 0.017, considered significant. All data were analyzed using the SAS software package.

Results

Effects of adjunctive antithrombotic agents on thrombolysis and acute reocclusion. Baseline left circumflex artery flow velocities were similar among the four treatment arms. The mean ($\pm$SD) time taken for occlusive thrombus to form was 73.0 ± 31.0 min (range 23 to 136). In the saline control group, the mean time to reperfusion was 32.4 ± 12.7 min. All three active treatment groups demonstrated accelerated thrombolysis compared with the control group (all p < 0.05, Table 1), with similar times to onset of reperfusion in each of the three active treatments.

Figure 1. Schematic showing the coronary patency for individual dogs. Time 0 indicates commencement of t-PA infusion. In the TFPI group, six dogs received the agent at the dose of 100 μg/kg per min, whereas the remaining four (*) received 150 μg/kg per min. In the DEGR VIIa group, seven dogs were given a bolus of 1 mg/kg, and the remaining three (*) received a 2-mg/kg bolus. All 10 dogs in the TAP group received the agent at a dose of 30 μg/kg per min (see text for details). Dog 7 in the TAP group died suddenly from ventricular fibrillation. Postmortem analysis of the circumflex artery demonstrated continued patency, without any macroscopic thrombus. CFVs = cyclic flow variations.
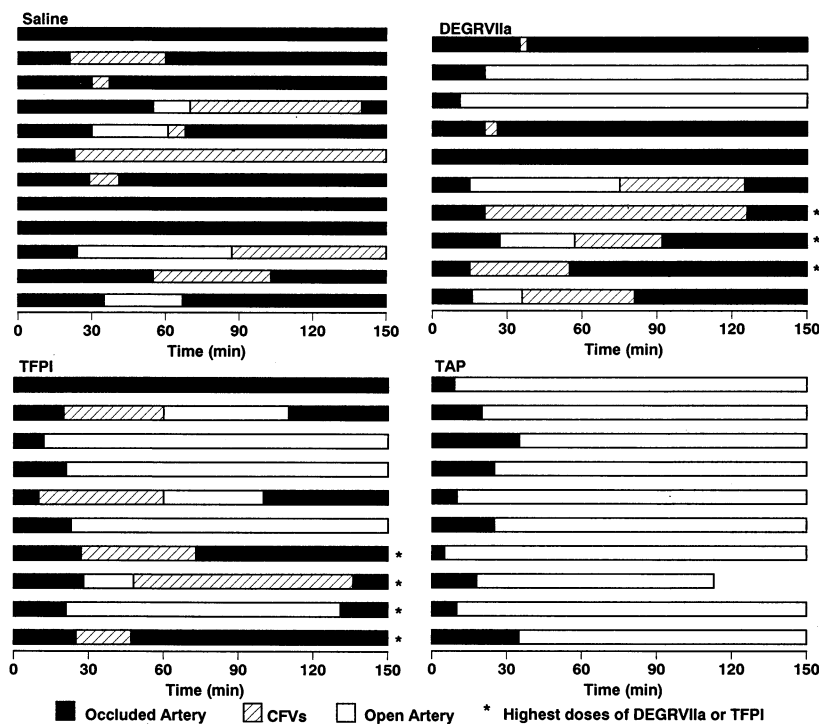
There were significant differences in the duration of reperfusion and incidence of cyclic flow variations and reocclusion among the three active treatment arms. The total duration of flow was greatest in the TAP-treated group. Compared with saline control, adjunctive TAP therapy resulted in an almost twofold increase in mean duration of flow (117 ± 8 min vs. 62 ± 45 min, p < 0.05, Table 1). In contrast, DEGR VIIa and TFPI therapy did not prolong the total duration of reperfusion compared with saline control. The duration of reflow at 100% or greater than the baseline flow rate—a marker of the integrity and efficiency of thrombolysis—was also greatest in TAP-treated dogs (117 ± 8 min with TAP vs. 47 ± 43 min with DEGR VIIa and 71 ± 50 min with TFPI, p = 0.0001). Both TAP (p = 0.0001) and TFPI treatment (p = 0.004) resulted in longer periods of flow at or greater than 100% of the baseline flow rate, compared with DEGR VIIa treatment, which was not significantly different from saline control.

The incidence of cyclic flow variations in the four treatment groups is shown in Table 1. Although DEGR VIIa and TFPI did not reduce the occurrence of cyclic flow variations compared with saline control, there were no instances of cyclic flow variations in TAP-treated dogs (p < 0.001 compared individually with TFPI, DEGR VIIa and control). Rates of reocclusion of the treated vessel are also shown in Table 1. There were no reocclusions in the TAP-treated group, and this reocclusion rate was significantly lower than with TFPI (p = 0.009) or DEGR VIIa (p = 0.002). Reocclusion rates of these last two treatments were not significantly different from saline control.

The doses of DEGR VIIa and TFPI were both increased after six experiments in each group, as prespecified in the study protocol, because observed reocclusion rates were ≥50% (four

of six dogs with DEGR VIIa and three of six dogs with TFPI). The dose of DEGR VIIa was increased from a bolus of 1.0 to 2.0 mg/kg, and the dose of TFPI was increased from a 90-min infusion at 100 to 150 μg/kg per min. There was no improvement in the efficiency of thrombolysis noted with the increased doses of either agent (Fig. 1). The mean time to reperfusion was unchanged for both agents compared with the lower doses, while reocclusion occurred in all dogs receiving the higher doses of both DEGR VIIa and TFPI.

Effects on hemostatic variables. The responses of PT and aPTT to the four study agents are shown in Figures 2 and 3, respectively. Tissue factor pathway inhibitor prolonged PT up to 7.4-fold above baseline, which was unexpected. In contrast, TAP and DEGR VIIa produced mild prolongation of PT and were not significantly different from saline control. No differences in aPTT response were noted among the four treatment arms. Gum BT was significantly prolonged by TAP (4.7 times above baseline, p = 0.004 compared with control) and TFPI (3.5 times above baseline, p = 0.003 compared with control). DEGR VIIa did not prolong gum BT to a greater extent than the saline control did (Fig. 4).

There were significant increases in FPA and TAT levels in the saline control group, particularly at the end of the t-PA infusion period, indicating heightened thrombin activity with thrombolysis. Fibrinopeptide A formation over time was suppressed with TFPI (p = 0.023) and TAP (p = 0.01) compared with control, whereas DEGR VIIa did not suppress FPA levels (Fig. 5). All three active agents suppressed TAT formation compared with control (p = 0.002 for all), although the effect was more marked in the TFPI- and TAP-treated groups (Fig. 6).
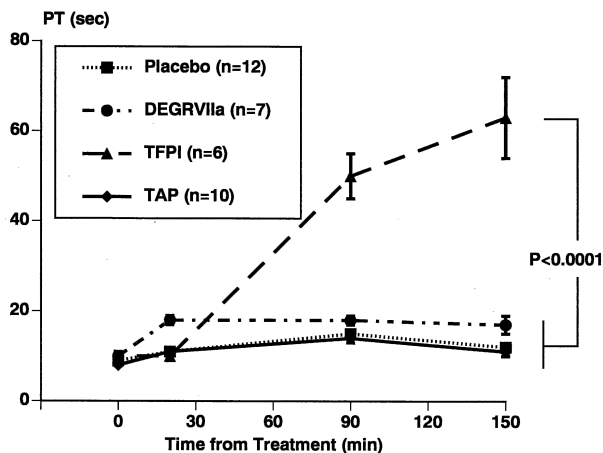
Figure 2. Graph illustrating PT values during administration of study agents. TFPI markedly prolonged PT up to 7.4 times above baseline. In contrast, TAP and DEGR VIIa did not change PT significantly, compared with the saline control.

Hemodynamic responses. No changes in heart rate during sinus rhythm were noted in any of the four treatment groups during the course of the experimental protocol. Baseline systemic blood pressure was similar among all four groups. There were no differences in blood pressure response with treatment with DEGR VIIa, TFPI or TAP compared with saline control.

TFPI and DEGR VIIa plasma levels. The mean TFPI plasma level concentrations, measured in six dogs before receiving TFPI, were undetectable. After administration of the dose of 100 μg/kg per min for 90 min, TFPI levels were 8.2 $\pm$ 5.5 μg/ml at 20 min, 15.6 $\pm$ 7.4 μg/ml at 90 min and 1.1 $\pm$ 1.3 μg/ml at 150 min.

Using an assay that does not detect canine factor VII, after a bolus injection of 1 mg (n = 7) or 2 mg (n = 3) of DEGR VIIa, the levels of DEGR VIIa were 6.9 $\pm$ 5.3 μg/ml and

Figure 3. Graph illustrating aPTT values during administration of study agents. None of the agents significantly prolonged aPTT compared with the saline control.
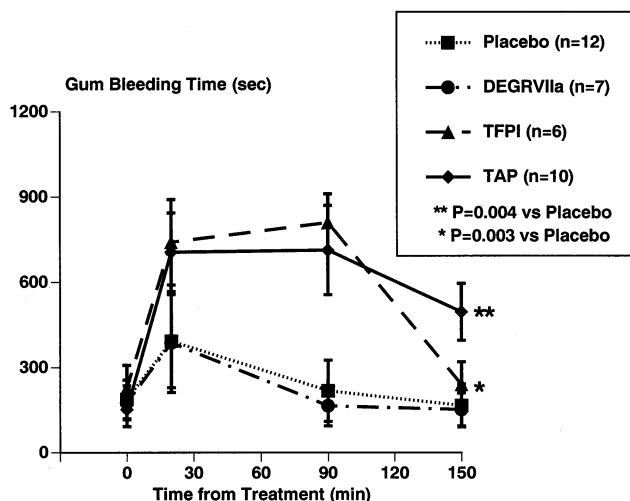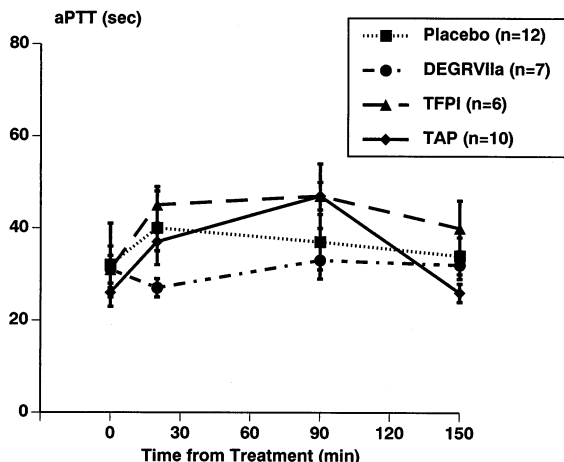

Figure 4. Plot showing the effect of study agents on gum bleeding time. DEGR VIIa did not prolong gum bleeding time compared with the saline control. In contrast, gum bleeding time was prolonged 4.7 times above baseline by TAP and 3.5 times above baseline by TFPI.

11.7 $\pm$ 06 μg/ml (at 1 and 2 mg, respectively) at 20 min, 4.1 $\pm$ 2.0 μg/ml and 6.3 $\pm$ 1.8 μg/ml at 90 min and 3.0 $\pm$ 1.3 μg/ml and 5.8 $\pm$ 0.7 μg/ml at 150 min.

Discussion

This study demonstrated that the efficiency of thrombolysis can be improved with adjunctive antithrombotic therapy targeted at the extrinsic coagulation pathway. The agents DEGR VIIa and TFPI both inhibit the factor VIIa–TF complex, whereas TAP selectively inhibits factor Xa. The efficacy of

Figure 5. Plot illustrating FPA values during administration of study agents. The FPA levels increased in the saline control group after thrombolytic therapy. In contrast, FPA formation was suppressed with both TFPI and TAP, whereas DEGR VIIa did not suppress fibrinopeptide A.
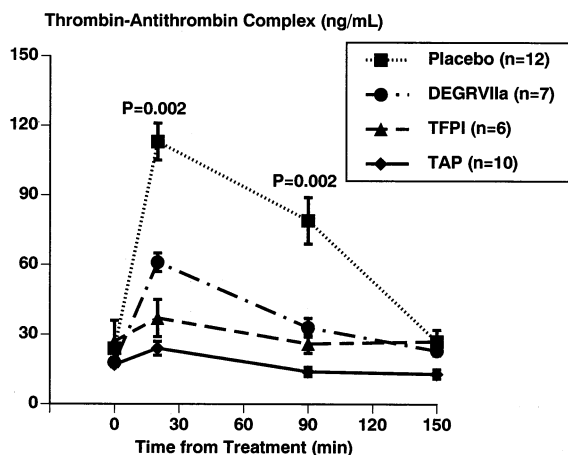
Thrombin-Antithrombin Complex (ng/mL)
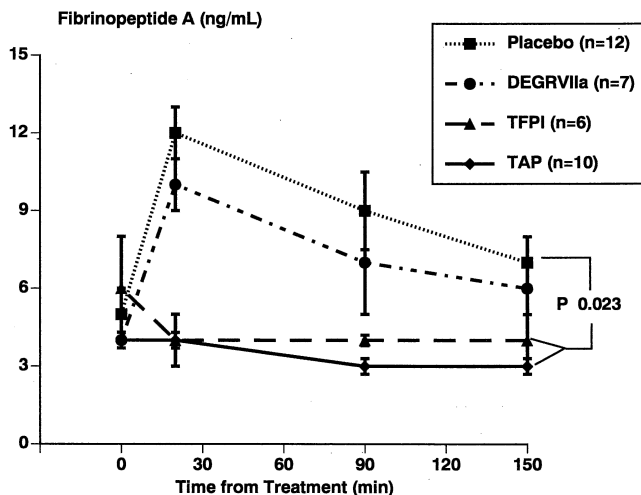
Figure 6. Plot illustrating TAT complex values during administration of study agents. The TAT complex increased in the saline control group after thrombolytic therapy. All three active agents suppressed TAT complex formation compared with saline control.

inhibition of the TF–factor VIIa–factor Xa pathway in accelerating thrombolysis (by an average of 12 min for all three active study agents) is consistent with the already established role of TF in initiating intravascular thrombosis (5,6). Yet, neither DEGR VIIa nor TFPI successfully prevented acute reocclusion, even at the higher doses tested.

It is possible that the lack of efficacy demonstrated with these two agents relates to the continuing catalytic effect of small amounts of factor VIIa–TF complex that escape blockade. The amplification process inherent in the early portion of the coagulation pathway facilitates formation of significant quantities of thrombin, even if only small amounts of factor VIIa–TF are present. Thus, virtually total inhibition of factor VIIa–TF complex may be necessary if this strategy is to be an effective adjunct to thrombolysis. Furthermore, factor Xa that is already formed has been found to contribute to rethrombosis (22,23), and is not controlled by factor VIIa–TF blockade.

In contrast, inhibition of factor Xa effectively prevented acute rethrombosis and was associated with the greatest suppression of markers of thrombin activity. Factor Xa is an integral component of both intrinsic and extrinsic coagulation pathways and is involved in several feedback loops that amplify the generation of thrombin, independent of factor VIIa–TF (24,25). The presence of factor Xa within the lysing clot has also been shown to have considerable procoagulant activity, not reliant on the presence of thrombin (23,26). Furthermore, plasmin generated by thrombolytic therapy acts paradoxically to stimulate thrombin formation (27) and can activate the contact system (28) and factor V (29). These procoagulant effects of plasmin are theoretically blocked by TAP, but not by agents that only inhibit factor VIIa–TF complex.

We observed that TAP administration abolished cyclic flow variations during reperfusion, whereas TFPI and DEGR VIIa treatments did not. All three study agents were tested in animals with a complex thrombus composed of platelets and fibrin (11). Cyclical build-up and embolization of platelet

thrombi have been suggested as mechanisms behind cyclic flow variations (30). It is noteworthy that inhibition of thrombin generation by TAP markedly reduces both fibrin and platelet deposition to the arterial surface after thrombolysis (31). In the present study, the morphology of the residual thrombus after treatment with either TFPI or DEGR VIIa was not assessed. Thus, further investigations are needed to determine whether the failure of inhibition of factor VIIa–TF complex to remove residual platelets is responsible for the cyclic flow variations and reocclusion in these experimental groups.

We observed varying effects on hemostatic variables among the three active agents. The modest elevation of PT associated with the factor Xa inhibitor, TAP, has been observed in a number of previous studies (3,32–34). Although DEGR VIIa resulted in similarly modest PT prolongation, TFPI markedly prolonged PT up to 7.4 times the baseline value. This finding is at variance with previous studies of TFPI in canine models (15,16). The explanation for these discrepant findings is unclear and will need to be addressed in future experiments with this agent. Prolongation of gum BT with TAP and TFPI, observed in this study, has been reported previously (16,33,35), although BTs in our study were substantially longer.

We measured markers of thrombin activity and correlated them with the study's in vivo findings. Fibrinopeptide A and TAT principally reflect thrombin activity and are only indirect measures of thrombin generation. The observation that these markers could be suppressed by agents that were otherwise ineffective in vivo (DEGR VIIa and TFPI) points to a relatively low sensitivity of FPA and TAT in discriminating significant alterations in thrombin generation. Unfortunately, direct measurement of thrombin generation by prothrombin fragment 1+2 cannot be determined in the dog with currently available assays.

Comparison with previous studies. A number of studies have previously demonstrated the benefit of TAP as an adjunct to thrombolysis (2–4,33–35). We and other investigators have compared TAP with the direct thrombin inhibitor, hirudin, in the canine thrombolytic model (2–4). Although hirudin was beneficial as an adjunct to thrombolysis in experimental and clinical studies (36–39), comparisons of TAP and hirudin have clearly shown that the potential utility of direct thrombin inhibition is significantly limited by its lack of suppression of ongoing thrombin generation (2–4).

Accordingly, our study went further to explore the effect of "higher tier" blockade of the coagulation pathway on the efficiency of thrombolysis. Haskel et al. (15) previously demonstrated that TFPI (previously known as lipoprotein-associated coagulation inhibitor) prevented reocclusion in a canine femoral artery model of platelet-rich thrombus. The same workers subsequently evaluated TFPI in a canine coronary artery model, similar to the one used in our study. In contrast to our results, they found that TFPI did not accelerate thrombolysis, but did improve reocclusion rates compared with control (16). Although these findings are difficult to reconcile with our own, differences in methodology and preparation of the thrombus may be responsible, and comparisons with other

extrinsic pathway inhibitors were not performed. In contrast to TFPI, DEGR VIIa has not been previously tested as an adjunct to thrombolysis. However, a monoclonal antibody directed against TF has been shown to inhibit thrombus formation (40,41) and improve thrombolysis (42) in a rabbit model. To our knowledge, our study is the first to compare the effects of three separate and distinct inhibitors of the extrinsic pathway on thrombolysis.

As the search for the most effective treatment for acute coronary thrombosis evolves, it appears that the efficacy of adjunctive agents that specifically inhibit thrombin activity, such as heparin and hirudin, may still be limited by permitting ongoing thrombin formation. This study has demonstrated that inhibition of thrombin generation by blockade of the extrinsic coagulation pathway, at one of several points, is effective at accelerating thrombolysis. However, selective inhibition of the strategically important coagulation protein, factor Xa, rather than blockade of factor VIIa–TF complex, provided the greatest protection against acute reocclusion. It is envisaged that on the basis of this and other studies, a refined adjunctive approach to thrombolytic therapy can be formulated that combines effective suppression of thrombin formation and inhibition of thrombin and platelet activity to achieve the ultimate goal of "optimal" reperfusion.

Study limitations. First, our study used an animal model of acute coronary thrombosis, and its results may not extend to coronary reocclusion occurring more than a few hours after thrombolysis. Second, dose-response studies with the various study agents were not performed during this experiment. However, preliminary studies were performed to determine the minimal dose of TAP required to produce suppression of markers of thrombin activity in the canine model (4). The dose of TAP chosen was significantly lower than in previous studies (2,3). Yet, this dose proved effective and did not cause excessive disturbance of hemostasis. The dose of TFPI was based on earlier dose-response studies, and the mean TFPI plasma concentrations obtained in our study are comparable with those previously published (15,16). We acknowledge that an insufficient dose of DEGR VIIa cannot be excluded as an explanation for the observed lack of efficacy and minimal effects on coagulation variables. However, the circulating levels of DEGR VIIa measured in this study are comparable to the levels found in a study demonstrating the efficacy of DEGR VIIa in preventing restenosis (13). This, together with the concordant results of the other extrinsic pathway inhibitor, TFPI, suggests that inadequate dosing is unlikely to be responsible for the observed lack of efficacy of DEGR VIIa.

References

1. Lincoff AM, Topol EJ. Illusion of reperfusion: does anyone achieve optimal reperfusion during acute myocardial infarction? Circulation 1993;87:1792–1805.
2. Sitko GR, Ramjit DR, Stabilito II, Lehman D, Lynch JJ Jr, Vlasuk GP. Conjunctive enhancement of enzymatic thrombolysis and prevention of thrombotic reocclusion with selective factor Xa inhibitor, tick anticoagulant peptide. Comparison to hirudin and heparin in a canine model of acute artery thrombosis. Circulation 1992;85:805–15.
3. Lynch JJ Jr, Sitko GR, Mellott MJ, et al. Maintenance of canine coronary artery patency following thrombolysis with front loaded plus low dose maintenance conjunctive therapy. A comparison of factor Xa versus thrombin inhibition. Cardiovasc Res 1994;28:78–85.
4. Nicolini FA, Lee P, Malycky JL, et al. Selective inhibition of factor Xa during thrombolytic therapy markedly improves coronary artery patency in a canine model of coronary thrombosis. Blood Coagul Fibrinolysis 1996;7:39–48.
5. Davie EW, Fujikawa K, Kisiel W. The coagulation cascade: initiation, maintenance and regulation. Biochemistry 1991;30:10363–70.
6. ten Cate H, Bauer KA, Levi M, et al. The activation of factor X and prothrombin by recombinant factor VIIa in vivo is mediated by tissue factor. J Clin Invest 1993;92:1207–12.
7. Vlasuk GP. Structural and functional characterization of tick anticoagulant peptide (TAP): a potent and selective inhibitor of blood coagulation factor Xa. Thromb Haemost 1993;70:212–6.
8. Broze GJ, Girard TJ, Novotny WF. Regulation of coagulation by a multivalent Kunitz-type inhibitor. Biochemistry 1990;29:7539–46.
9. Rapaport SI. The extrinsic pathway inhibitor: a regulator of tissue-factor dependent blood coagulation. Thromb Haemost 1991;66:6–15.
10. Nicolini FA, Mehta JL, Nichols WW, Saldeen TG, Grant M. Prostacyclin analogue iloprost decreases thrombolytic potential of tissue-type plasminogen activator in canine coronary thrombosis. Circulation 1990;81:1115–22.
11. Nicolini FA, Nichols WW, Saldeen TG, Mehta JL. Pathological basis of failure of concurrent glyceryl trinitrate therapy to improve efficacy of tissue type plasminogen activator in coronary thrombosis. Cardiovasc Res 1991;25:283–9.
12. Romson JL, Haack DW, Lucchesi BR. Electrical induction of coronary artery thrombosis in the ambulatory canine, a model for in vivo evaluation of antithrombotic agents. Thromb Res 1980;17:841–53.
13. Jang Y, Guzman LA, Lincoff AM, et al. The influence of blockade at specific levels of the coagulation cascade on restenosis in a rabbit atherosclerotic femoral artery injury model. Circulation 1995;92:3041–50.
14. Lindahl AK, Wildgoose P, Lumsden AB, et al. Active site-inhibited factor VIIa blocks tissue factor activity and prevents arterial thrombus formation in baboons [abstract]. Circulation 1993;88 Suppl I:I–417.
15. Haskel EJ, Torr SR, Day KC, et al. Prevention of arterial reocclusion after thrombolysis with recombinant lipoprotein-associated coagulation inhibitor. Circulation 1991;84:821–7.
16. Abendschein DR, Meng YY, Torr-Brown S, Sobel BE. Maintenance of coronary patency after fibrinolysis with tissue factor pathway inhibitor. Circulation 1995;92:944–9.
17. Diaz-Collier JA, Palmier MO, Kretzmer KK, et al. Refold and characterization of recombinant tissue factor pathway inhibitor expressed in *Escherichia coli.* Thromb Haemost 1994;71:339–46.
18. Vlasuk GP, Ramjit D, Fujita T, et al. Comparison of the in vivo anticoagulant properties of standard heparin and the highly selective factor Xa inhibitors antistasin and tick anticoagulant peptide (TAP) in a rabbit model of venous thrombosis. Thromb Haemost 1991;65:257–62.
19. Nicolini FA, Lee P, Rios G, Kottke-Marchant K, Topol EJ. Combination of platelet fibrinogen receptor antagonist and direct thrombin inhibitor at low doses markedly improves thrombolysis. Circulation 1994;89:1802–9.
20. Novotny WF, Brown SG, Miletich JP, Rader DJ, Broze GJ. Plasma antigen levels of the lipoprotein-associated coagulation inhibitor in patient samples. Blood 1991;78:387–93.
21. Jolley ME, Wang CH, Ekenberg SJ, Zuelke MS, Kelso DM. Particle concentration fluorescence immunoassay (PCFIA): a new, rapid immunoassay technique with high sensitivity. J Immunol Methods 1984;67:689–93.
22. Eisenberg PR, Siegel JE, Abendschein DR, Miletich JP. Importance of factor Xa in determining the procoagulant activity of whole-blood clots. J Clin Invest 1993;91:1877–83.
23. Prager NA, Abendschein DR, McKenzie CR, Eisenberg PR. Role of

thrombin compared with factor Xa in the procoagulant activity of whole blood clots. Circulation 1995;92:962–7.

24. Neuenschwander P, Jesty J. A comparison of phospholipids and platelets in the activation of human factor VIII by thrombin and factor Xa, and the inactivation of factor Xa. Blood 1988;72:1761–70.

25. Warn-Cramer BJ, Rapaport SI. Evidence suggestive of activation of the intrinsic pathway of blood coagulation after injection of factor Xa/ phospholipid into rabbits. Arterioscl Thromb Vasc Biol 1995;15:133–9.

26. Eisenberg PR, Sherman L, Rich M, et al. Importance of continued activation of thrombin reflected by fibrinopeptide A to the efficacy of thrombolysis. J Am Coll Cardiol 1986;7:1255–62.

27. Eisenberg PR, Miletich JP, Sobel BE. Factors responsible for the differential procoagulant effects of diverse plasminogen activators in plasma. Fibrinolysis 1991;5:217–24.

28. Ewald GA, Eisenberg PR. Plasmin-mediated activation of contact system in response to pharmacological thrombolysis. Circulation 1995;91:28–36.

29. Lee CD, Mann KG. The activation of human coagulation factor V by plasmin. Blood 1989;73:185–90.

30. Nicolini FA, Lee P, Rios G, Zajkowski Brown J, Plow EF. Selective inhibition of factor Xa and the prothrombinase complex prevents coronary rethrombosis [abstract]. Circulation 1994;90 Suppl I:I–334.

31. Folts JD, Gallagher K, Rowe GG. Blood flow reductions in stenosed canine coronary arteries: vasospasm or platelet aggregation? Circulation 1982;65:248–54.

32. Schaffer LW, Davidson JT, Vlasuk GP, Siegl PKS. Antithrombotic efficacy of recombinant tick anticoagulant peptide, a potent inhibitor of coagulation factor Xa in a primate model of arterial thrombosis. Circulation 1991;84:1741–8.

33. Mellott MJ, Holahan MA, Lynch JJ Jr, Hasuk GP, Dunwiddie CT. Acceleration of recombinant tissue-type plasminogen activator-induced reperfusion and prevention of reocclusion by recombinant antistasin, a selective factor Xa inhibitor in a canine model of femoral arterial thrombosis. Circ Res 1992;70:1152–60.

34. Benedict C, Ryan J, Todd J, et al. Active site-blockade factor Xa prevents thrombus formation in the coronary vasculature parallel with inhibition of extravascular coagulation in a canine thrombosis model. Blood 1993;81:2059–66.

35. Mellott MJ, Stranieri MT, Sitlo GR, Stabilito II, Lynch JJ Jr, Vlasuk GP. Enhancement of recombinant tissue plasminogen activator–induced reperfusion by recombinant tick anticoagulant peptide, a selective factor Xa inhibitor, in a canine model of femoral arterial thrombosis. Fibrinolysis 1993;7:195–202.

36. Mruk JS, Zoldhelyi P, Webster MW, et al. Does antithrombotic therapy influence residual thrombus after thrombolysis of platelet-rich thrombus? Effects of recombinant hirudin, heparin, or aspirin. Circulation 1996;93:792–9.

37. Haskel EJ, Prager NA, Sobel BE, Abendschein DR. Relative efficacy of antithrombin compared with antiplatelet agents in accelerating coronary thrombolysis and preventing early reocclusion. Circulation 1991;83:1048–56.

38. Neuhaus KL, von Essen R, Tebbe U, et al. Safety observations from the pilot phase of the randomized r-hirudin for improvement of thrombolysis (HIT-III) study. A study of the Arbeitsgemeinschaft Leitender Kardiologischer Krankenhausarzte (ALKK). Circulation 1994;90:1638–42.

39. Cannon CP, McCabe CH, Henry TD, et al. A pilot trial of recombinant desulfatohirudin compared to heparin in conjunction with tissue plasminogen activator and aspirin for acute myocardial infarction. Results of the Thrombolysis in Myocardial Infarction (TIMI) 5 trial. J Am Coll Cardiol 1994;23:993–1003.

40. Pawasche A, Golino P, Ambrosio G, et al. A monoclonal antibody against rabbit tissue factor inhibits thrombus formation in stenotic injured rabbit carotid arteries. Circ Res 1994;74:56–63.

41. Jang I, Gold H, Leinbach R, Fallon J, Collen D, Wilcox J. Antithrombotic effect of a monoclonal antibody against tissue factor in a rabbit model of platelet mediated arterial thrombosis. Arterioscler Thromb 1992;12:948–54.

42. Ragni M, Cirillo P, Pascucci I, et al. Monoclonal antibody against tissue factor shortens tissue plasminogen activator lysis time and prevents reocclusion in a rabbit model of carotid artery thrombosis. Circulation 1996;93:1913–8.