The Profibrinolytic Effect of Activated Protein C in Clots Formed From Plasma Is TAFI-Dependent

By Laszlo Bajzar, Michael E. Nesheim, and Paula B. Tracy

Thrombin-activatable fibrinolysis inhibitor (TAFI) is the precursor of an exopeptidase that is identical to plasma carboxypeptidase B. Upon activation by thrombin, activated TAFI (TAFIa) attenuates fibrinolysis, presumably by catalyzing the removal of C-terminal lysines from partially degraded fibrin. Activated protein C (APC) proteolytically inactivates the essential cofactor in prothrombinase, factor Va, and limits both the formation of thrombin and subsequent activation of TAFI, thereby appearing profibrinolytic. APC is able to reconstitute an APC-dependent shortening of lysis time in a purified system; however, it remained to be determined the extent to which TAFI is involved in the profibrinolytic effect of APC in a plasma-based system. To aid in addressing this question, two monoclonal antibodies (MoAbTAFM16 and #13) and a polyclonal antibody were produced against purified TAFI. MoAbTAFM16 was shown to inhibit TAFI activation and thereby appears to stimulate fibrinolysis. Furthermore, an enzyme-linked immunosorbent assay was developed using MoAbTAFM13 and the polyclonal antibody. Through its use, the plasma concentration of TAFI was determined to be 73 nmol/L. In addition, a turbidity assay was used to determine the effect of APC on tissue plasminogen activator-induced fibrinolysis of clots produced from normal human plasma (NHP), plasma immunodepleted of TAFI (TdP), and TdP reconstituted with purified TAFI. APC shortened lysis time of clots produced from NHP in a saturable and concentration-dependent manner. However, APC had no effect on lysis time of clots formed from either TdP or NHP in the presence of 80 nmol/L MoAbTAFM16. The APC effect could be reconstituted in TdP by the addition of purified TAFI. The lysis time in TdP was increased from 50 to 180 minutes in a TAFI concentration-dependent manner. The EC₅₀ was 15 nmol/L and saturation was approached at physiologically relevant concentrations (60 nmol/L). The profibrinolytic effect of APC was also compared with that of MoAbTAFM16 and two competitive inhibitors, an inhibitor of the carboxypeptidase A and B family purified from potato tubers and 2-Guanidinoethylmercaptoacetic acid (GEMSA). All were able to reduce lysis time of clots formed from normal human plasma by 90 minutes, yielding respective EC₅₀ values of 5 nmol/L, 15 nmol/L, 50 nmol/L, and 90 nmol/L. Therefore, the majority of the profibrinolytic effect of APC, in an in vitro plasma system, is dependent on TAFI. Because TAFI dramatically influences lysis time, inhibitors of TAFI or TAFI activation may prove to be important adjuvants for thrombolytic therapy.

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modulin. Therefore, the anticoagulant pathway (thrombin, thrombomodulin, and protein C), the antifibrinolytic pathway (thrombin, thrombomodulin, and TAFI), and the distribution of thrombin between these pathways could play a key role in providing a link between coagulation and fibrinolysis and maintaining a balance among these cascades.

Because APC has been reported to potentiate fibrinolysis5-11 and we have shown that APC is only able to promote fibrinolysis in the presence of the terminal step of the coagulation cascade involving prothrombinase-dependent prothrombin activation,7 it is reasonable to hypothesize that, in a plasma system, APC enhances fibrinolysis by inhibiting the formation of thrombin and preventing the activation of TAFI. Although TAFI suffices to reconstitute a profibrinolytic effect of APC in a system of purified components,10 the requirement for TAFI in the profibrinolytic effect of APC in clots formed from plasma has not been shown. Therefore, the studies detailed here were undertaken to determine unequivocally whether APC exerts its profibrinolytic effect in clots formed from plasma through a TAFI-dependent mechanism.

The results indicate that APC does not affect lysis of clots formed from plasma in the presence of a monoclonal antibody (MoAbTAFI#16) that prevents the activation of TAFI or the lysis of clots formed from plasma depleted of TAFI. However, the profibrinolytic effect of APC was reconstituted in TAFI-deficient plasma supplemented with TAFI. The ability of APC to promote fibrinolysis was compared with that of inhibitors of the carboxypeptidase B family and that of MoAbTAFI#16. Each profibrinolytic component appeared identically profibrinolytic; however, both APC and MoAbTAFI#16 were found to be most potent. We, therefore, propose that APC promotes fibrinolysis of clots formed from plasma by preventing the formation of thrombin during fibrinolysis, thereby preventing activation of TAFI and subsequent inhibition of fibrinolysis.

MATERIALS AND METHODS

Materials. Recombinant t-PA, Activase, was provided by Dr G. Vehar of Genentech (South San Francisco, CA). The lyophilized powder was dissolved in water to give a final concentration of 1.0 mg/mL, from which working stock solutions were prepared as described previously.16 Plasma (Lot No. 91531) was purchased from George King Biomedical (Overland Park, KS). A soluble form of thrombomodulin, Solulin, was provided by Dr J. Morser of Berlex (Richmond, CA). The sheep polyclonal anti-TAFI antibody was provided by H. Hoogendoorn of Affinity Biologicals (Hamilton, Ontario, Canada). 2-Guanidinoethylmercaptosuccinic acid (GEMSA), a specific inhibitor of the carboxypeptidase B-like family, was purchased from Calbiochem (La Jolla, CA). Potato tuber inhibitor (PTI), an inhibitor of carboxypeptidase A and B-like exopeptidases, was purchased from Sigma (St Louis, MO). Horseradish peroxidase (HRP) was purchased from Boehringer Mannheim (Laval, Quebec, Canada). PCPS vesicle (PC:PS 3:1) were prepared according to the method of Barenholz et al.18 Prothrombin was purified as described previously16 and used to prepare thrombin by a modification of the procedure of Lundblad et al,19 as described previously.20 Protein C was purified and then activated by incubation with thrombin in the presence of EDTA, as described previously.4 TAFI was purified as previously described.10 TAFI was radiolabeled with 125I using Iodo-beads (Pierce, Rockford, IL) as directed by Pierce. Free and bound 125I were separated on Sephadex G-10 equilibrated in 10 mmol/L Tris, 0.15 mol/L NaCl, pH 7.4 (TBS) containing 1% bovine serum albumin (BSA-TBS). The column was developed with TBS. Fractions containing 125I-labeled TAFI were diluted 1:1 with glycerol and stored at -20°C.

Production, characterization, and purification of the anti-TAFI MoAbs designated MoAbTAFI#13 and #16. Female, 6-week-old BALB/c mice were immunized intraperitoneally with 75 µg of purified TAFI in complete Freund's adjuvant. Booster injections of 25 µg TAFI in incomplete Freund's were administered on days 16 and 37 after the initial injection. On day 66, the mice received booster injections of 25 µg TAFI in the absence of adjuvant. Three days later, the mice were killed and the isolated spleen cells were fused to murine myeloma NS-1 cells27 using polyethylene glycol as described by Ol.22 Hybridoma cells were selected using hypoxanthine, aminopterin, and thymidine and grown in culture as described previously.22 Fourteen days after fusion, hybridoma cells producing anti-TAFI MoAbs were identified using a solid-phase radioimmunomossay by capturing anti-TAFI antibodies from conditioned media with immobilized goat antimouse IgG followed by quantitation of specifically bound 125I-labeled TAFI. Hybridomas testing positive for antibodies against TAFI were subcloned by limiting dilution and retested. Ascites for two hybridomas, designated MoAbTAFI#13 and MoAbTAFI#16, were prepared in pristane-primed BALB/c mice by injecting 1 x 10⁶ cells/mouse.24 Concentrates enriched in MoAbTAFI#13 and #16 were produced from ascites fluid by gel filtration on S-200 and concentrated with (NH₄)₂SO₄. The pellets were dissolved in 50% glycerol/H₂O and stored at 4°C.

Immobilization of MoAbTAFI#16 to Sepharose 4B. The concentrated MoAbTAFI#16 solution was diluted and dialyzed against 0.1 mol/L sodium citrate, pH 6.5, and then coupled to CNBr-activated Sepharose 4B (Pharmacia, La Jolla, CA) by modification of the procedure of Cuatrecasas,25 as reported previously.26 Two milliliters of 2.5 mg/mL MoAbTAFI#16 were produced from ascites fluid by gel filtration on S-200 and concentrated with (NH₄)₂SO₄. The pellets were dissolved in 50% glycerol/H₂O and stored at 4°C.

Affinity purification and HRP conjugation of polyclonal anti-TAFI. The caprylic acid precipitated sheep antibodies were subjected to affinity purification by passing the antibodies dialyzed against 20 mmol/L HEPES, 150 mmol/L NaCl, pH 7.4 (HBS) over a 1.0 mL Sepharose CL-4B to which 1.0 mg of TAFI had been coupled. TAFI was coupled using the protocol described for immobilization of MoAbTAFI#16. Antibodies retained on the affinity column were eluted using gentle Elution Buffer (Pierce). After extensive dialysis against PBS, affinity-purified antibodies were concentrated using an Amicon concentrator with a 20 kDa filter (Amicon, Oakville, Ontario, Canada), diluted by the addition of an equal volume of PBS, and stored at -20°C. HRP was conjugated to the polyclonal anti-TAFI IgG using a periodate coupling method. The HRP-coupled polyclonal anti-TAFI was precipitated with the addition of (NH₄)₂SO₄ to 50% saturation and pelleted by centrifugation. The pellet was washed twice by resuspension in 50% saturated (NH₄)₂SO₄/H₂O followed by centrifugation. The final pellet was dissolved in a volume of 0.5% glyceral/H₂O to yield a 1.0 mg/mL solution of HRP-conjugated polyclonal anti-TAFI, which was stored at -20°C.

Development of an enzyme-linked immunoassay (ELISA) for quantification of TAFI. Wells of a 96-well microtiter plate, Corning (Corning, NY), were coated with MoAbTAFI#13 by incubating 10 µg/mL MoAbTAFI#13 in 50 mmol/L sodium carbonate, pH 9.6, for 2 hours at 22°C. Nonspecific sites were blocked with BSA. Samples were diluted in 0.1% BSA in PBS with 0.1% Tween 20 and 100 µL of each sample was applied to each well and incubated for 1.5 hours at 22°C. HRP-conjugated anti-TAFI was then incubated
with each sample and specific binding was determined o-phenylene-
diamine dihydrochloride (Sigma). The color was allowed to develop
for 10 minutes. The reaction was then quenched by the addition of
sulfuric acid to each well. Absorbance was measured at 490 nm and
corrected by subtracting the absorbance at 650 nm using a Ther-
nomax microtiter plate reader (Molecular Devices, Sunnyvale, CA).
Using this ELISA, the concentration of TAFI in a plasma pool (≥60
donors) was determined to be 73 nmol/L, which compares favorably
with a concentration of 50 nmol/L that was calculated based on
recovery of activity during purification. Concentrations in plasma
obtained from 6 volunteers ranged from 50 to 175 nmol/L.

Western blotting. Samples were diluted in sample preparation
buffer (1% sodium dodecyl sulfate [SDS], 1% 2-mercaptoethanol
[BME], and 10% glycerol with bromophenol blue) and subjected to
SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using a 5% to
15% gradient gel in a mini-slab gel electrophoresis apparatus
(Hoefer, Technical Marketing, Ottawa, Ontario, Canada) according
to the procedure of Neville.27 Protein was then transferred to Nitro-
cellulose (Schleicher & Schuell, Keene, NH) at 500 mA for 1.0 hour
using the electroblotting techniques described by Towbin et al.28
Nitrocellulose was blocked with 5% nonfat dry milk in 20 mmol/L
Tris, 0.15 mol/L NaCl, 0.05% Tween 20 at pH 7.4 (TBS-T). TAFI
antigen was probed for 1.0 hour with MoAbTAFI#13, which was
dissolved in TBS-T to a concentration of 0.1 µg/mL. MoAbTAFI#13
was probed for 1.0 hour with an HRP-conjugated antianimal
IgG (Southern Biotechnologies, Birmingham, AL) diluted in 5,000
in TBS-T. The secondary antibody was detected by enhanced chemi-
luminescence (Chemiluminescence Detection Kit; DuPont, Boston,
MA) using X-Omat film (Kodak Scientific Imaging) and an M35A
X-Omat processor (Eastman Kodak, New Haven, CT).

Preparation of plasma for lysis assays. Pooled (3 to 4 donors)
fresh frozen human plasma was purchased from Haematologic Tech-
nologies Inc (Burlington, VT) and dialyzed against 4 L of HBS, at
4°C with 4 changes overnight. The dialysate, termed normal human
plasma (NHP), was stored at −70°C in 50 mL aliquots. TAFI-
deficient plasma (TDP) was produced by passing an aliquot, maxi-

mally 50 mL, of NHP over a 2.0-mL anti-TAFI affinity column
(MoAbTAFI#16-Sepharose 4B) equilibrated in HBS. Depletion of
TAFI was verified by Western blot analysis of a sample from each
individual fraction (data not shown). Only the flow-through fractions
depleted of TAFI with an equal to the NHP loaded onto the
column were pooled and subsequently stored in 2.0-mL aliquots at
−70°C. Pooled plasma samples were also subjected to Western blot-
ing and TAFI concentrations were quantified by ELISA. Figure 1
shows the signal associated with a serial dilution of either a preclu-
ning or postcolumn sample as detected using ELISA. The signal
detected using the ELISA described above, Specificity of MoAbTAFI#13 is shown by Western blot analy-
sis, as shown in the inset. Chemiluminescence using MoAbTAFI#13
and HRP-conjugated antianimal IgG distinguished a band in NHP
that exhibited an identical electrophoretic mobility to purified TAFI
(compare lanes 1 and 3). However, this band is not present in plasma
that had been passed over immobilized MoAbTAFI#16 (lane 2).
These data indicate that the affinity column comprising MoAb-
TAFI#16 is able to deplete TAFI from plasma. Furthermore, quanti-
tation of TAFI by ELISA indicates that a single pass of plasma over
the affinity column resulted in the removal of 99.9% of TAFI from
plasma.

Lysis assay. The assay was performed by initially diluting the
plasma, either NHP or TDP, with HBS such that the A590 equal to the
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APC shortens lysis time of clots formed from normal hu-
man plasma. A turbidometric lysis assay was used to show
the effect of APC on tPA-induced lysis of clots formed from
normal human plasma. NHP dialyzed and diluted one-third
with HBS was allowed to clot in the presence of thrombin,
Ca2+, PCPS vesicles, tPA, and various concentrations of
APC (0 to 30 nmol/L). Clots were maintained at 37°C and
and thrombin (8.0 nmol/L final concentration) in the presence and
absence of MoAbTAFI#16 (12.5 µmol/L) for 10 minutes at 22°C.
Thrombin was then inhibited by the addition of Phe-Pro-Arg chlo-
romethyl ketone (FPA-CK; 50 mmol/L final concentration). A nega-
tive control was produced by adding FPA-CK before initiation of
the reaction by the addition of TAFI. The effect of MoAbTAFI#16 on
TAFIa was determined by the addition of the antibody subsequent
to the activation of TAFI. The volume was corrected in each of the
other 3 reactions by the addition of a volume of buffer equal to that
of the added MoAbTAFI#16. A 25-µL aliquot was removed from
each of the 4 reactions (30 µL final concentration) and added to 975
µL of hippuryl arginine (410 µmol/L) in HBS with 0.01% Tween 80.
The time course of hippuric acid formation at 22°C was followed by
monitoring absorbance at 254 nm and 0.5-minute intervals using a
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RESULTS

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puryl arginine (Fig 3). No TAFIa activity was generated when either MoAbTAFI#16 or FPA-CK, a specific inhibitor of thrombin, was present during the incubation of TAFI with thrombin and thrombomodulin. However, the addition of MoAbTAFI#16 subsequent to activation was without effect on TAFIa activity, because the rate of hippuryl arginine hydrolysis was identical to the control rate. These data indicate that MoAbTAFI#16 does not inhibit TAFIa, but does prevent the activation of TAFI.

The profibrinolytic effect of APC is abrogated by MoAbTAFI#16. The effect of MoAbTAFI#16 on tPA-induced lysis of clots formed from normal human plasma was investi-
TAFI and the Profibrinolytic Effect of APC

The antifibrinolytic effect of TAFI can be overcome in at least three ways: by preventing the formation of its activator, thrombin; by preventing the activation of TAFI; and by inhibiting TAFI. Therefore, the efficacy of APC, an inhibitor of thrombin formation; MoAbTAFI#16, an inhibitor of TAFI activation; and GEMSA and PTI, inhibitors of carboxypeptidase B-like enzymes, to inhibit the TAFI-dependent antifibrinolytic effect on lysis time was investigated. The TAFI-dependent profibrinolytic effect of each compound was evaluated by determination of lysis time of clots formed from both NHP and TdP in the presence of various concentrations of each inhibitor. APC, GEMSA, and MoAbTAFI#16 at every concentration studied did not affect lysis time of clots formed from TdP. In general, the lysis time remained fairly constant at approximately 50 minutes, indicating that none of the inhibitors used exhibited TAFI-dependently increases in clot lysis times was completely inhibited by 50 nmol/L APC, because the lysis time in the presence of APC remained invariant at 50 minutes over the range of TAFI concentrations studied. These data indicate that the profibrinolytic effect of APC is not apparent in TdP; however, it can be reconstituted in TdP by the addition of purified TAFI. Therefore, the profibrinolytic effect of APC in clots formed from plasma, as quantified using a turbidometric lysis assay, is due solely to a mechanism involving TAFI. The data also indicate that as little as 3 nmol/L TAFI is required to observe an APC-dependent profibrinolytic effect. Furthermore, the TAFI-dependent prolongation of lysis time approaches saturation with 60 nmol/L TAFI, which is within the physiologic range of concentrations determined both immunologically (see the Materials and Methods) and is similar to the concentration calculated based on recovery of activity during purification.

Comparison of APC to inhibitors of carboxypeptidase B-like proteases (GEMSA and PTI) and MoAbTAFI#16 in their ability to enhance lysis time of clots formed from human plasma. The antifibrinolytic effect of TAFI can be overcome in at least three ways: by preventing the formation of its activator, thrombin; by preventing the activation of TAFI; and by inhibiting TAFI. Therefore, the efficacy of APC, an inhibitor of thrombin formation; MoAbTAFI#16, an inhibitor of TAFI activation; and GEMSA and PTI, inhibitors of carboxypeptidase B-like enzymes, to inhibit the TAFI-dependent antifibrinolytic effect on lysis time was investigated. The TAFI-dependent profibrinolytic effect of each compound was evaluated by determination of lysis time of clots formed from both NHP and TdP in the presence of various concentrations of each inhibitor. APC, GEMSA, and MoAbTAFI#16 at every concentration studied did not affect lysis time of clots formed from TdP. In general, the lysis time remained fairly constant at approximately 50 minutes, indicating that none of the inhibitors used exhibited TAFI-dependently increases in clot lysis times was completely inhibited by 50 nmol/L APC, because the lysis time in the presence of APC remained invariant at 50 minutes over the range of TAFI concentrations studied. These data indicate that the profibrinolytic effect of APC is not apparent in TdP; however, it can be reconstituted in TdP by the addition of purified TAFI. Therefore, the profibrinolytic effect of APC in clots formed from plasma, as quantified using a turbidometric lysis assay, is due solely to a mechanism involving TAFI. The data also indicate that as little as 3 nmol/L TAFI is required to observe an APC-dependent profibrinolytic effect. Furthermore, the TAFI-dependent prolongation of lysis time approaches saturation with 60 nmol/L TAFI, which is within the physiologic range of concentrations determined both immunologically (see the Materials and Methods) and is similar to the concentration calculated based on recovery of activity during purification.
A shortening of lysis time in the absence of TAFI could not be shown for any component; however, all compounds saturably shorten lysis of clots formed from NHP by 90 minutes. The data indicate that APC most potently enhanced fibrinolysis and is followed by PTI and GEMSA, respectively. Although the magnitude of the profibrinolytic effect was 90 minutes for each inhibitor, the ECSO associated time of clots formed from TdP for every concentration of MoAbTAFI#16, PTI, and GEMSA, respectively.

The lysis time at saturating concentrations of each inhibitor exhibited a minimum lysis time (average, 50 minutes) that is identical to the lysis time of clots formed from NHP by 90 minutes. The data indicate that APC most potently enhanced fibrinolysis and is followed by MoAbTAFI#16, PTI, and GEMSA, respectively.

DISCUSSION

In an attempt to understand the influence of APC on fibrinolysis, numerous studies have been performed. These studies have shown a profibrinolytic effect of APC both in vitro, in systems ranging from whole blood to purified blood components, and in vivo, in the dog and cat model. APC has been shown to form complexes with plasminogen activator inhibitor-I (PAI-1), an inhibitor of tPA. In addition, preincubation of PAI-1 with APC before its addition to plasma attenuates the inhibitory effect of PAI-1 on lysis of clots formed from that plasma. It was proposed that APC consumes PAI-1, facilitating an increased rate of plasminogen activation. However, this mechanism has been questioned for two reasons. The first reason is that complexes of APC and PAI-1 form relatively slowly and the kinetics of inactivation of PAI-1 by APC do not support the conclusion that APC would effectively compete for PAI-1 in the presence of tPA. The second reason is that complexes of APC and PAI-1 can be shown in the absence of an effect on fibrinolysis in systems comprising whole blood and APC from heterologous species. We were able to show that the profibrinolytic effect of APC in clots formed from human plasma is due directly and specifically to its ability to inhibit prothrombin activation. These data rationalize the observations of others who have shown that APC promotes lysis in normal plasma but not factor X- or VIII-deficient plasma, that other anticoagulants are also able to potentiate fibrinolysis, and that APC from a specific species is profibrinolytic only in those species in which it is also an anticoagulant. Inclusion of TAFI in a system composed of purified components, including a negatively charged surface, Ca++, factors Va and Xa, and prothrombin was able to reproduce an APC-dependent profibrinolytic effect. However, those studies did not show that the mechanism of APC-dependent profibrinolytic effect in clots formed from purified components was identical to that which is observed in plasma.

This study shows that the effect of APC on fibrinolysis of clots formed from plasma is exclusively dependent on TAFI. Although APC can shorten lysis time in a saturable manner (Fig 2), it is unable to shorten lysis time in the presence of an antibody raised against TAFI, MoAbTAFI#16 (Fig 4). Because APC had no effect on lysis time in the presence of MoAbTAFI#16, it is unlikely that the thrombin generated during the lysis assay in the absence of APC had any secondary effect, such as changing clot structure, that could affect lysis time. MoAbTAFI#16 is able to shorten lysis time in a saturable manner and to an extent similar to that of APC. The identical lysis times achieved in the presence of saturating amounts of either APC or MoAbTAFI#16 suggest a common mechanism involving inhibition of the activation of TAFI. APC indirectly inhibits activation of TAFI by preventing the formation of thrombin and the data in Fig 3 show that MoAbTAFI#16 directly inhibits activation of TAFI by interacting with TAFI. The interaction of the antibody with TAFI most likely precludes the interaction of TAFI with thrombin. This mechanism is suggested because the model for activation of TAFI by the complex of thrombin and thrombomodulin includes only the binary interactions involving thrombin and TAFI or thrombin and thrombomodulin but not between thrombomodulin and TAFI.

The requirement for TAFI in the profibrinolytic effect of APC in plasma was verified further by immunodepleting TAFI from plasma and showing that APC does not influence the lysis of clots formed from this plasma. Western blotting analyses and an ELISA developed during this study were used to show that the plasma was indeed depleted of TAFI (Fig 1). Although APC was without effect on lysis of clots formed from this plasma, supplementation of the plasma with purified TAFI both prolonged lysis time of the TdP in the absence of APC and restored the APC-dependent profibrinolytic effect (Fig 5). Saturation with respect to prolongation of lysis time occurred at a physiologically relevant concentration of TAFI (60 nmol/L).
The profibrinolytic effect of APC was compared with that of the specific inhibitor of TAFI activation, MoAbTAFI#16, and the general inhibitors of the carboxypeptidase B-like family, GEMSA and PTI (Fig 6). The magnitude of the effect of each reagent was identical, although they differed with respect to their potency (APC>>MoAbTAFI#16>>PTI->>GEMSA). Cumulatively, these data indicate that TAFI plays a major antifibrinolytic role in clots formed from plasma under conditions in which substantial prothrombin activation occurs. In this system, APC potentiates fibrinolysis but only in a TAFI-dependent manner. Therefore, we propose that APC appears profibrinolytic primarily by inhibiting the production of thrombin, thereby preventing the activation of TAFI and its subsequent inhibition of fibrinolysis. A model is represented in Fig 7.

It is apparent that coagulation, fibrinolysis, anticoagulants, and, by inference, antifibrinolics are not likely to work independently, as exemplified by the presented data. In the system used, the initial clot size was constant. Furthermore, 100% of the fibrinogen in the plasma was converted to fibrin; thus, fibrin accretion could not have occurred. Therefore, neither variation in clot size nor fibrin accretion could have affected lysis time. Because the same plasma was used for all experiments, variations in lysis times resulting from differing levels of inhibitors of fibrinolysis, specifically PAI-1, also cannot account for the profibrinolytic effect of APC. Therefore, the data indicate that the state of the coagulation system during fibrinolysis can affect the fibrinolytic system. This is borne out by the observation that patients deficient in factor XI tend to bleed in areas of high fibrinolytic potential and, as speculated by von dem Borne et al,9 may be the result of insufficient thrombin generation to satisfactorily stabilize the clot. Inhibiting the coagulation cascade, therefore, could render a fibrin clot more susceptible to degradation as a result of a lack of activated TAFI. A physiologic example in which this may be of therapeutic benefit is in the treatment of a myocardial infarction due to thrombus. It is a current belief that treatment of myocardial infarction by tPA may be more efficacious by combination therapy.42 Inclusion of an anticoagulant might inhibit the activation of TAFI and subsequently destabilize the fibrin clot rather than, or in addition to, preventing further clot formation. If TAFI is found to affect thrombolysis in this situation, then inhibitors of activated TAFI should be considered as new avenues of thrombolytic therapy. As shown, using an in vitro clot lysis assay, both APC and inhibitors of either activated TAFI or TAFI activation enhance lysis and would reduce the amount of tPA required to achieve a lysis time equal to that which would be obtained in their absence. Removal of thrombi, under conditions in which inhibition of coagulation or induction of fibrinolysis is either impossible or undesired, may be accomplished by the prevention of TAFI activation or inhibition of activated TAFI, thereby allowing the endogenous fibrinolytic system to work more efficiently. Alternatively, specific activators of TAFI may augment replacement therapies or maintain hemostasis in individuals whose coagulation system is compromised. The effect of the TAFI pathway on the human fibrinolytic system has not been investigated in vivo. However, because an effect of APC on fibrinolysis has been shown both in vivo, in dog30 and cat models,31 and in vitro, in a human plasma model, TAFI likely will be found to play a physiologic role in the balance between coagulation and fibrinolysis in humans.

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