Prevention of coronary thrombosis with subthrombolytic doses of tissue-type plasminogen activator*

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ABSTRACT To determine whether tissue-type plasminogen activator (t-PA) may prevent coronary thrombosis or accelerate the lysis of clot formed under conditions in which increased concentration of the activator is present before thrombosis, clot lysis studies were undertaken in vitro and in vivo. In vitro, exogenous t-PA (6 to 100,000 ng/ml) accelerated the lysis of clot in a dose-dependent fashion when the clot was formed either from whole plasma or from euglobulin fractions (n = 316 determinations). Adding t-PA before clot formation shortened the time to lysis by at least threefold with euglobulin fractions and by at least 10-fold with whole plasma clots, which is consistent with the presence of inhibitors of fibrinolysis in whole plasma and with the binding of t-PA to nascent fibrin. In an intact dog preparation of coronary thrombosis (n = 25), occlusive thrombus formation was prevented when t-PA was present in subthrombolytic concentrations (430 to 1200 ng/ml, n = 5). Occlusive thrombus formation occurred after only discontinuation of the t-PA infusion and clearance of t-PA. Lower concentrations of t-PA (147 to 427 ng/ml, n = 6) significantly delayed occlusion (26 ± 6.5 vs. 7.8 ± 2.8 min for controls). In animals with t-PA concentrations of less than 140 ng/ml (n = 4), the time to occlusion was unaltered (7.7 ± 4.5 min). The present study demonstrates that t-PA present before clot formation inhibits thrombosis or accelerates thrombolysis depending on concentration, and that subthrombolytic doses of t-PA can prevent thrombus formation in vivo. The findings of this study provide a rationale for clinical strategies aimed at augmenting t-PA concentrations in patients at high risk for coronary thrombosis or at risk for reocclusion after thrombolysis.


WE HAVE RECENTLY demonstrated that tissue-type plasminogen activator (t-PA) is an effective agent for the induction of coronary thrombolysis in experimental animals and in patients.1–4 Either intravenous1–4 or intramuscular5 administration of t-PA can lyse established thrombi. However, despite successful lysis of macrovascular clot, salutary effects on the ischemic heart depend on minimization of the delay before reperfusion.6–8 Thus, attention has focused on early implementaion of thrombolysis so that myocardial perfusion can be restored before irreversible injury in jeopardized tissue is complete.

Minimization of the duration of coronary occlusion is facilitated by the use of agents that are effective after systemic rather than intracoronary administration. Recently, we have shown that effective blood levels of t-PA can be achieved promptly when t-PA is administered intramuscularly in association with an absorption-enhancing agent.5 Although the levels of t-PA attainable are lower than those achieved with intravenous administration, they are of the same order of magnitude that has been demonstrated to be effective in inducing thrombolysis in experimental studies.

An additional strategy could involve the identification of patients at high risk for occlusion or reocclusion and pretreatment of incipient thrombosis. Patients presenting with unstable angina and high-grade critical lesions are at increased risk for coronary occlusion,
and in patients in whom successful thrombolysis has
been achieved, residual high-grade coronary lesions
predispose to reocclusion despite administration of an-
ticoagulants or antiplatelet agents.4, 9–11

In view of these considerations, the present study
was performed to determine whether t-PA can prevent
coronary thrombosis or can accelerate thrombolysis of
clots that form under conditions in which an increased
concentration of the activator is already present. Its
rational depends in part on the kinetic properties of
t-PA, including its high affinity for fibrin and its facili-
tated activation of plasminogen within clot as opposed
to in the circulation.12–15

The key properties of t-PA that distinguish it from
conventionally available pharmacologic activators of
the fibrinolytic system include its high fibrinolytic ac-
tivity,12, 14 despite a short half-life in the circulation,2, 16
coupled with only modest induction of proteolytic ac-
tivity in the circulating blood when t-PA is adminis-
tered in therapeutically effective doses.3, 4, 11, 17 t-PA
has a high affinity for fibrin. The fibrin–t-PA complex
in turn has a high affinity for plasminogen. The Mi-
ichaelis constant (Km) of t-PA for circulating free plas-
minogen in plasma is much higher (approximately 65
μM) than the Km of fibrin-bound t-PA for plasminogen
(0.16 μM).12, 13 Accordingly, little or no conversion
of circulating plasminogen (generally 2 μM) to plasmin
occurs despite activation of plasminogen at the fibrin
surface.14, 18 Thus, plasmin is formed locally
without marked fibrinogenolysis or proteolysis in the
systemic circulation.

To characterize dose-response relationships for lysis
of clot by t-PA when the agent was present before clot
formation in comparison with those when the activator
added after clot formation, we measured clot dissolu-
tion in euglobulin and plasma fractions in vitro with
exogenous t-PA over a wide range of concentrations.
In contrast to clots formed in plasma, those formed in
euglobulin fractions contain diminished levels of fi-
brinolytic inhibitors and, primarily, α2-antiplasmin.
Previous investigations have not characterized this re-
lationship in plasma clots over concentration ranges
that may be applicable in vivo for rapid coronary
thrombolysis. An additional aim of this study was
to determine whether pretreatment in vivo with sub-
thrombolytic doses of t-PA, before induction of coro-
nary thrombi, could prevent or retard coronary
thrombosis. Accordingly, infusions of t-PA were
administered to dogs before induction of occlusive
thrombus with a copper coil introduced under fluo-
rescopic guidance into the coronary artery. This ex-
perimental animal preparation has been characterized ex-
tensively in studies of more than 300 animals in our
laboratory.

Methods

Studies in vitro. Venous blood was obtained without the use of a tourniquet from 10 healthy male volunteers on two different
occasions. It was collected into citrated Vacutainer tubes at 0°
to 4°C, with a final concentration of citrate-10 mM. Plasma was
harvested at 4°C by centrifugation for 10 min at 2000 g and
stored at −70°C until assay.

For studies in vitro, clot was formed by the addition of throm-
bin and clot lysis time was measured as the interval required for
total dissolution of a clot. Clot lysis in vitro was assessed under
the four following sets of conditions, in samples with a concen-
tration range of t-PA of 6 to 100,000,000 ng/ml; (1) "prethrombosis"
conditions with whole plasma in which exogenous t-PA was
added before formation of clot and lysis time was compared
with that occurring spontaneously in samples in which no ex-
ogenous t-PA had been added, (2) "postthrombosis" conditions
with whole plasma in which exogenous t-PA was added 5 min
after coagulation had been induced, (3) "prethrombosis" condi-
tions with euglobulin fractions of plasma samples in which
t-PA was added before formation of clot, and (4) "postthrombo-
sis" conditions with euglobulin fractions in which exogenous
t-PA was added 5 min after clot had been formed. Prethrombo-
sis conditions were used to permit maximal incorporation of
t-PA into interstices of the clot while it was being formed. Post-
thrombosis conditions were used to mimic conditions in vivo
in which only endogenous t-PA would have maximal access to
the interstices of a fibrin clot being formed, with exogenous t-PA
available to penetrate the clot after it was established.

The t-PA used (Genentech, Inc., lot BH001DAX) was pro-
duced by recombinant DNA technology in a mammalian cell
culture system.

The euglobulin fraction of plasma contains fibrinogen, plas-
minogen, and endogenous plasminogen activator. However, it
contains reduced levels of most of the plasma inhibitors of
fibrinolysis such as α2-antiplasmin. Euglobulin fractions were
prepared from thawed plasma samples by diluting 0.5 ml ali-
quots with 9.5 ml of distilled water at 4°C, adjustment of pH to
5.9 with acetic acid, centrifugation for 10 min at 3000 g at 4°C,
removal of the supernatant fraction, and solubilization of the
precipitate in 0.5 ml of imidazole-buffered saline (0.025M imi-
dazole HCl, 0.113M NaCl, pH 7.4), containing 0.8 mg/ml bovine serum albumin.

Clots were induced in vitro by addition of 50 μl of a solution
containing 0.5 IU of freshly prepared thrombin in imidazole-
buffered saline to 0.2 ml aliquots of either whole plasma or
euglobulin fractions in 10 mm × 75 mm clear-glass test tubes at
melting ice-bath temperature. After immediate mixing and for-
mation of clot, tubes were incubated at 37°C. The interval
required for clot lysis was assessed visually and recorded as the
time required for complete dissolution of clot. Lysis time
was expressed in units defined by Marsh,19 i.e., $10^6$(lysis time in
min)$^2$. Either t-PA or buffered saline vehicle was added in 20 μl
aliquots either before or 5 min after addition of thrombin to yield
a final concentration of 6 to 100,000 ng/ml, approximately
equivalent to the range of 10 to 200,000 IU/ml for the Interna-
tional Reference Preparation of t-PA (IRP-t-PA, used for cali-
bration). Fibrinogen content was assayed in pooled plasma by
the method of Claus.20 For each set of conditions, assays at
each concentration were performed in quadruplicate and each
set of conditions was reassessed with a second batch of pooled
plasma from the same subjects.

Studies in vivo
Animal preparations. Twenty-five mongrel dogs weighing
23 to 35 kg were anesthetized with 12.5 mg/kg of intravenously administered thiopental and 60 mg/kg α-chloralose after premedication with 1 mg/kg morphine sulfate given subcutaneously. After a cuffed endotracheal tube had been inserted, ventilation was maintained with room air and a Harvard respirator. Catheters were placed in the abdominal aorta and vena cava via the left femoral artery and vein and flushed with normal saline without heparin. A canula was placed in a peripheral vein of the right leg of each animal for administration of 50 μg/kg/min lidocaine in saline. The left common carotid artery was isolated for introduction of coronary artery catheters and insertion of a copper coil was used to induce coronary thrombosis. Coronary arteriography was performed with meglumine diatrizoate with the use of a Siemens radiographic unit.

For the induction of coronary thrombi, a copper coil, 5 mm in length, was inserted into the left anterior descending coronary artery. The coil was formed by wrapping a copper wire (0.5 mm diameter) in a spiral around a 19-gauge needle, as previously described. With the animal in the right anterior oblique position, a modified USCI No. 1 Amplatz coronary catheter was inserted selectively into the left anterior descending coronary artery under fluoroscopic guidance. A Teflon-coated guidewire, 0.021 inch in diameter, was inserted, and the angiographic catheter was removed. The copper coil was advanced into the artery over the guidewire with the use of a narrow gauge (0.025 inch internal diameter, 0.038 inch external diameter) Formacath catheter, the guide wire was removed, and the coil was lodged in the midportion of the left anterior descending coronary artery. The Formacath catheter was withdrawn slightly to a position 1 cm proximal to the coil.

**Experimental protocol.** Thirty minutes after baseline coronary angiography had been performed, control blood samples were obtained. These and subsequent samples were drawn into ice-cold 5 ml Vacutainer tubes containing sodium citrate for assay of fibrinolytic activity and immunoradiometrically detectable human t-PA. After collection at 0° to 4°C, samples were centrifuged at the same temperature. Plasma was frozen promptly and stored at −70°C before assay within 2 weeks. To rapidly attain constant plasma concentrations of exogenous t-PA, infusions were initiated with a bolus injection of t-PA providing 7.7 times the amount subsequently infused per minute. This dosage was based on considerations of blood levels anticipated, the known rates of clearance of plasma in dogs defined in preliminary studies, and the calculations outlined in the Appendix. An initial sample was obtained at zero time, before administration of t-PA. The next sample was obtained 10 min after onset of administration of t-PA. The thrombogenic copper coil was placed in the left anterior descending coronary artery immediately after acquisition of the second sample. Subsequent samples were obtained at frequent intervals throughout the infusion of t-PA.

t-PA was infused at one of the following rates: 1 to 1.75 μg/kg/min, n = 4; 2.5 μg/kg/min, n = 6; 3.75 μg/kg/min, n = 4; 5.0 μg/kg/min, n = 2; and 10.0 μg/kg/min, n = 1. Control dogs (n = 8) underwent angiography and coil insertion, but did not receive exogenous t-PA. The time to coronary occlusion was recorded in each case, and the electrocardiogram was monitored throughout for detection of ischemia and reperfusion arrhythmia. For continuing evaluation of coronary patency, low-dose injections of coronary contrast were given and fluoroscopy was performed at 5 min intervals. When electrocardiographic criteria of ischemia or fluoroscopic criteria of occlusion appeared, thrombotic occlusion was confirmed by repeat cineangiography.

Infusion of t-PA was discontinued 70 min after its initiation. Subsequent blood samples were obtained at 5 min intervals for an additional 40 min for determination of the rate of clearance of t-PA from the circulation. In those animals in which coronary occlusion occurred before the end of the infusion, the infusion was stopped, and sampling for determination of clearance of t-PA was initiated immediately after acquisition of the confirmatory angiogram. In those animals in which coronary patency was maintained throughout the entire infusion of t-PA, repeat fluoroscopy was performed during the interval in which clearance of t-PA from the circulation was being assessed. During the interval in which plasma concentrations of t-PA were declining, the time of occurrence of coronary occlusion induced by the indwelling coronary thrombogenic coil was identified.

**Assay of plasma samples.** t-PA antigen was assayed with a two-site immunoradiometric assay, as previously described. The procedure involves binding of t-PA to anti-t-PA adsorbed to wells in a microtiter plate and subsequent binding of radiolabeled [125I]-anti-t-PA to bound t-PA. After removal of excess [125I]-anti-t-PA, the amount of bound [125I]-t-PA is determined with a gamma counter. Canine t-PA does not cross react in the immunoradiometric assay system used. Anti-t-PA antiserum and purified human melanoma t-PA for the reference standards were provided by Professor Désiré Collen.

t-PA functional activity was assayed conventionally with fibrin plates as follows: Circular zones of fibrinolysis were measured by planimetry on fibrin plates exposed to serial dilutions of euglobulin fractions of samples. These were prepared by dilution of citrated plasma (1:20) with distilled water, adjustment to pH 5.6 with acetic acid, centrifugation, and solubilization of precipitates in imidazole-buffered saline (pH 7.4) containing 0.8% bovine serum albumin. Fibrin plates were prepared with fibrinogen (Kabi Diagnostica), thrombin (Sigma), and CaCl2 (0.05M), and were incubated at 37°C for 16 hr. Zones of lysis were quantified by comparison with those induced with IRP-t-PA.

**Results**

**Studies in vitro.** Pooled plasma was found to contain 1.9 mg/ml fibrinogen and 7.5 ng/ml of endogenous t-PA, equivalent to approximately 14 U/ml of IRP-t-PA cocolibrated with the fibrin-plate assay. Lysis of clot in euglobulin fractions and in whole plasma was assessed in 316 samples with exogenous t-PA in the sample in concentrations ranging from 0 to 100,000 ng/ml.

As shown in figure 1, clot lysis was accelerated in a concentration-dependent fashion in response to t-PA in whole plasma. For each concentration, when t-PA was present before clot formation, more rapid lysis of clot occurred than was the case when the same concentration of t-PA was present only after clot formation.

As shown in figure 2, clots formed from euglobulin fractions of plasma exhibited more prompt lysis at a given concentration of t-PA than those formed in whole plasma because of the diminished quantities of inhibitors of components of the fibrinolytic system in euglobulin fractions compared with those in plasma.

With addition of t-PA after thrombin-coagulation in euglobulin fractions and in whole plasma samples, the response to a given concentration of exogenous t-PA was diminished (figure 2) compared with that which

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CIRCULATION
FIGURE 1. Studies in vitro. Relationship between the rate of lysis and concentration of t-PA. Results are expressed as a percentage of the maximal rate of lysis. The results shown are for whole plasma samples in which exogenous t-PA was present at the time of clot formation (pretreatment) or after clot formation (posttreatment). Administration of t-PA before, in comparison with after, clot formation resulted in a shift of the dose-response curve to the left such that lower concentrations of t-PA were required to achieve similar rates of lysis. The concentration of t-PA that achieved the maximal rate of lysis as a result of pretreatment achieved only 70% of the maximal rate of lysis under posttreatment conditions. The results are consistent with the influence of plasma inhibitors in the posttreatment group and with different access of t-PA to established compared with nascent clot.

FIGURE 2. Studies in vitro. Data for the four groups of conditions are shown. Values are means ± SD. Pretreatment and posttreatment refer to the groups in which exogenous t-PA was present at the time of clot formation or added after formation. The euglobulin fractions exclude the majority of circulating inhibitors. The results demonstrate that for either whole plasma or euglobulin fractions, the presence of t-PA at the time of clot formation results in substantially shorter times to lysis in comparison with those when the same concentrations of t-PA are added after clot formation. At the lowest concentrations of administered t-PA, lysis did not occur for up to 1500 min in the pretreatment whole plasma (t-PA concentrations less than or equal to 3.75 ng/ml) or posttreatment whole plasma groups (t-PA concentrations less than or equal to 12.5 ng/ml).
TABLE I
Concentrations (ng/ml) of t-PA required to achieve 50% of the maximal rate of lysis in vitro

<table>
<thead>
<tr>
<th>Time of addition of t-PA to whole plasma</th>
<th>Time of addition of t-PA to euglobulin fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before clot Subsequent to clot</td>
<td>Before clot Subsequent to clot</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>2670</td>
<td>6800</td>
</tr>
<tr>
<td></td>
<td>260</td>
</tr>
<tr>
<td></td>
<td>630</td>
</tr>
</tbody>
</table>

Results are for t-PA antigen activity (n = 316 analyses).

occurred when t-PA was present during clot formation. The displacement to the right of the dose-response curve for t-PA was consistent with decreased penetration of t-PA into established as opposed to nascent thrombi and the probable influence of inhibitors of t-PA and plasmin in the sample when t-PA was added after clot formation. In contrast, when t-PA is bound to fibrin, activation of plasminogen occurs at the fibrin surface and in the absence of the majority of plasmin inhibitors.

Table 1 summarizes the concentrations of t-PA required for acceleration of clot lysis to 50% maximum. To elicit lysis at comparable rates, only 40% as much t-PA was needed before clot formation in comparison with that needed after clot formation. The findings pertained to both euglobulin fractions and whole plasma studies.

Studies in vivo. Twenty-five dogs were studied. Eight were controls and 17 were given infusions of t-PA. Two animals given t-PA sustained ventricular fibrillation almost immediately after insertion of the coronary coil but before thrombotic coronary occlusion. They were excluded. Angiographically documented occlusive thrombus formed in control dogs in an average of 7.8 ± 2.5 (SD) min. Coronary angiograms documented induction, localization, and dissolution of coronary thrombi in each case. An example is shown in figure 3. The distribution of persistent clot was confirmed by dissection of each of the coronary arteries at autopsy.

Because the immunoradiometric assay used is specific for human t-PA, endogenous canine t-PA was not detected. Hence, values in all baseline samples and all samples from control dogs were zero. The fibrin-plate assay detects functional fibrinolytic activity whether it is attributable to endogenous canine or exogenous human t-PA or other activators. Thus, baseline samples exhibited fibrinolytic activity equivalent to 16.8 ± 8.8 IU/ml (range 7 to 35 IU/ml). Sequential samples in control dogs exhibited values indistinguishable from baseline values.

Bolus administration followed by constant infusion of t-PA elicited plateaus of concentrations of t-PA within 10 min (figure 4). Close concordance was observed between determinations of t-PA antigen and determinations of functional, fibrin plate–assayable activity at both higher and lower doses (figure 4). Clearance of either t-PA antigen or functional activity after cessation of infusion of t-PA conformed to a biexponential function with fast and slow components. The apparent disappearance rates exhibited considerable variability for both early and late components.

FIGURE 3. Three angiograms obtained in the right anterior oblique projection from a dog in which t-PA was administered by constant infusion before insertion of the thrombogenic copper coil. A, Control angiogram obtained before initiation of t-PA infusion. Distal left anterior descending artery (LAD) is patent. B, Angiogram obtained 60 min after copper coil insertion (arrow) during t-PA infusion at a rate of 5 µg/kg/min (t-PA concentration 761 ± 23 ng/ml). Patency of the distal LAD is demonstrated. C. Repeat angiogram from the same animal taken 40 min after discontinuation of infusion (plasma t-PA concentration 90.5 ng/ml). Total occlusion of the vessel is demonstrated at the site of the coil. Occlusive thrombus was confirmed by dissection of the vessel after the animal was killed.
FIGURE 4. t-PA antigen and fibrinolytic activity in animals that received a constant infusion of 5 µg/kg/min (A) or 1 µg/kg/min (B). The concordance of fibrinolytic and t-PA antigen activity is apparent. Whereas the artery of the animal in A did not occlude until 40 min after discontinuation of the infusion, that of the animal in B occluded 5.3 min after insertion of the coil. Clearance of t-PA antigen and fibrinolytic activity was determined by assessing samples after discontinuation of the infusion.

Thus, the half-time of disappearance (t1/2) ranged from 6.6 to 16.6 min for the fast component and from 26.8 to 56 min for the slow (late) component.

Steady-state infusions of t-PA precluded apparent clot formation when prevailing concentrations of t-PA in plasma exceeded 430 ng/ml. This was achieved with doses of 3.75 to 10 µg/kg/min (n = 5). When prevailing plasma levels of t-PA ranged from 147 to 427 ng/ml, the onset of coronary occlusion was significantly delayed (n = 6). Under these conditions, occlusion occurred 26 ± 6.5 min after insertion of the thrombogenic coil in four dogs, but was precluded throughout the duration of infusion in two. For animals given the lowest dose of t-PA, less than 1.75 µg/kg/min (n = 4), the onset of coronary occlusion was not delayed compared with that in control dogs (7.7 ± 4.5 min). Prevailing concentrations of t-PA antigen in plasma and corresponding functional activity results are shown in table 2.

In each of four animals in which coronary patency was maintained throughout the infusion, the time of onset of occlusion after cessation of infusion was compared with the simultaneous plasma concentration of t-PA while plasma t-PA was declining. Occlusion oc-
TABLE 2  
t-PA antigen and functional activity and time to coronary occlusion

<table>
<thead>
<tr>
<th>Infusion rate of t-PA (μg/kg/min)</th>
<th>t-PA antigen (ng/ml)</th>
<th>Fibrinolytic activity (IU)</th>
<th>Time to occlusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-10 (n = 3)</td>
<td>610-1198</td>
<td>78-152</td>
<td>&gt;60</td>
</tr>
<tr>
<td>3.75 (n = 2)</td>
<td>467-609</td>
<td>81-122</td>
<td>&gt;60</td>
</tr>
<tr>
<td>2.5 (n = 2)</td>
<td>165-387</td>
<td>65-108</td>
<td>&gt;60</td>
</tr>
<tr>
<td>1-1.7 (n = 4)</td>
<td>147-427</td>
<td>33-128</td>
<td>18-33</td>
</tr>
<tr>
<td>Zero (control) (n = 8)</td>
<td>129-150</td>
<td>29-123</td>
<td>5-13</td>
</tr>
</tbody>
</table>

Results are expressed as the range of values for each set of conditions.

Discussion

Results of studies in vitro demonstrate that for clots in which t-PA is incorporated at the time of clot formation, lysis is more rapid than for similar clots exposed to equivalent concentrations of t-PA only after clot is formed. Results with euglobulin fractions indicate that reduction of concentrations of inhibitors of the fibrinolytic system shifts the dose-response curve for t-PA such that less activator is necessary to elicit lysis at a comparable rate. These results are consistent with those of earlier studies suggesting that inhibitors of fibrinolysis are not concentrated in clots. Brommer has incubated plasminogen activator with clot before or after induction of coagulation in vitro. However, in contrast to his findings, we observed a close correlation between lysis time and the concentration of t-PA when activator was added after as well as before clot formation. Concentrations of t-PA necessary to elicit lysis were higher than those employed by Brommer, who used washed clots, possibly with limiting amounts of native plasminogen.

Zammaron et al. showed that elevations in concentration of exogenous t-PA accelerate thrombolysis. However, the dose range was limited and the endpoint used was clot dissolution over a 5 hr interval. In our study conditions, were consistent with the rapidity of lysis required and temporal constraints associated with coronary thrombolysis.

A major inhibitor of plasmin present in plasma but not present in euglobulin fractions, α2-antiplasmin, rapidly inactivates circulating plasmin (t1/2 = approximately 100 msec). In addition, plasma, but not euglobulin fractions, has been found to contain rapidly acting ("fast inhibitors") inhibitors of t-PA. Our findings, which indicate that the fibrinolytic activity of t-PA is diminished in whole plasma as compared with euglobulin fractions, are consistent with these observations.

A concentration of t-PA of 3560 ng/ml was required to induce a half-maximal rate of lysis of clots formed from whole plasma samples. Acceleration of thrombolysis resulted from pretreatment of the samples even when exogenous t-PA was present in concentrations as low as 6.25 ng/ml (figures 1 and 2). Although results in vitro cannot be extrapolated directly to conditions in vivo, they indicate the likelihood of substantial augmentation of the rate of fibrinolysis in vivo over a wide range of concentrations of t-PA by exposure of nascent as opposed to established clots to exogenous t-PA. Furthermore, they confirm that augmentation of clot lysis is readily accomplished in whole plasma samples containing inhibitors.

The results of our studies in vivo indicate that concentrations of t-PA in plasma that are too low to induce lysis of established clots are nevertheless capable of preventing or retarding coronary thrombosis. In clinical studies, coronary thrombolysis has been accomplished with elevation of plasma t-PA to concentrations of 1000 to 5000 ng/ml. In previous studies from our laboratory with the same experimental preparation as was used here, thrombolysis was elicited in dogs with elevation of plasma t-PA to concentrations in excess of 1000 ng/ml. In the present study, coronary thrombosis was prevented when concentrations of t-PA in plasma were in the range of 430 to 1200 ng/ml. Significant delay of the onset of coronary occlusion was demonstrated with even lower concentrations (147 to 427 ng/ml). Nevertheless, it should be appreciated that achievement of blood levels of this magnitude requires pharmacologic administration of exogenous t-PA and is not yet possible by utilization of any known stimulus to release or inhibition of catabolism of endogenous t-PA.

The efficacy of subthrombolytic concentrations of t-PA for prevention of coronary thrombosis may reflect the high affinity of t-PA for fibrin, the increased affinity of fibrin-bound t-PA for plasminogen, and the relative protection of t-PA and plasmin bound to fibrin from inhibitors. In addition, previous studies indicate that nascent fibrin with incomplete cross-linkage is likely to be more susceptible to lysis. The resistance to lysis of cross-linked α-chains of fibrin may
represent a rate-limiting factor of fibrinolysis of mature clot. 25

In contrast to t-PA, neither streptokinase nor urokinase binds selectively to fibrin. 27, 28 In clinical studies in which reduced doses of these agents have been administered, systemic proteolysis nevertheless occurred. 29-31 Thus, with either streptokinase or urokinase it appears unlikely that inhibition of clot formation will occur in vivo without induction of systemic proteolysis.

Several clinical speculations may be warranted. Clinically effective coronary thrombolysis must be implemented as soon as possible after the onset of myocardial ischemia if appreciable functional and metabolic recovery of myocardium is to be elicited. If incipient thrombosis can be recognized promptly and if a relatively clot-selective activator can be administered with dispatch, benefit may be maximized. Patients with unstable angina with high-grade coronary lesions and a high risk of coronary thrombosis may be shown ultimately to benefit from prevention of coronary occlusion with low doses of t-PA before angioplasty or coronary surgery. An obvious advantage of use of low-dose regimens in these settings is the avoidance of activation of the fibrinolytic system in circulating blood, which might otherwise compromise patients requiring immediate invasive or surgical intervention. Patients with high-grade residual stenoses that predispose to early reocclusion 9 after initially successful thrombolysis and for whom antiplatelet agents and systemic anticoagulation may not provide suitable protection 9, 11, 17 may be particularly benefited during the interval before bypass surgery or angioplasty. Despite the relatively fibrin-specific properties of t-PA and the lower doses required for prevention compared with dissolution of established coronary thrombi, prolonged administration of t-PA in inappropriately high doses can result in depletion of circulating \( \alpha_2 \)-antiplasmin and fibrinogenolysis. Nevertheless, our results suggest that approaches designed to modestly augment concentrations of t-PA in plasma before or concomitantly with the onset of coronary thrombosis may be useful. Although the experimental animal preparation used in our study does not necessarily simulate thrombosis in an atherosclerotic human coronary artery, the results obtained provide a framework for hypotheses readily testable in clinical investigations.

The potential for application of alternative methods for activation of fibrinolysis may merit reappraisal. Several agents, such as the oral anabolic steroid stanozolol, and the vasopressin analog 1-desamino-8-\( \alpha \)-arginine vasopressin (DDAVP), have been shown to raise fibrinolytic activity and increase endogenous t-PA activity. 32, 33 Stanozolol appears also to diminish the concentration of the fast inhibitor of t-PA, thereby increasing t-PA activity. 34 In the context of pretreatment of incipient thrombosis, this potential may be considerable. Concentrations of t-PA insufficient to induce lysis of established clot may nevertheless inhibit or prevent clot formation.

We thank Joanne Markham for assistance with the mathematical derivations used in the appendix, Amelia Grace and Denise Nachowiak for technical assistance with assays of fibrinolysis, Richard Rodriguez for assistance with the studies in animals, and Barbara Donnelly and Lori Dales for preparation of the typescript.

References


Appendix: Derivation of formula for determination of appropriate t-PA bolus

Judging from results in studies of dogs, in which we found that the t½ for the first phase of t-PA from plasma was on the order of 5.5 min, a peak concentration of t-PA in plasma was achieved within 30 min with a constant input:

\[ a = \frac{\ln 2}{t^{1/2}} = 0.126/min \] and \[ V \frac{dC(t)}{dt} = I - aVC(t) \] (1)

where \( a \) = elimination rate constant ng/min\(^{-1}\); \( t^{1/2} \) = half-time in plasma of t-PA (min); \( V \) = volume of distribution of t-PA (ml); \( I \) = constant infusion rate (t-PA ng/min); \( C(t) \) = concentration in plasma of t-PA at any time (t-PA ng/min).

Solving equation 1:

\[ C(t) = \frac{I}{Va} (1 - e^{-at}) \] (2)

assuming that the peak concentration is reached in 30 min.

\[ C_p = \frac{I}{Va} (1 - e^{-30a}) = 7.754 \frac{I}{V} min \] (3)

where \( C_p \) = desired peak concentration of t-PA in plasma (ng/ml).

For a bolus injection of \( B \) units of t-PA at time zero, the concentration in plasma at any time \( t \) is given by \( C_b(t) \):

\[ C_b(t) = \frac{B}{Ve^{at}} \] (4)

where \( B \) = bolus injection of t-PA at time zero (ng of t-PA). When the initial concentration at time 0 is equal to \( C_p' \):

\[ C_b(0) = C_p = \frac{B}{V} = 7.754 \frac{I}{V} min, \]

\[ B = 7.754 I min \] (5)

Therefore, an amount of t-PA equivalent to 7.754 times the amount given by infusion per minute is required for the bolus to rapidly attain a plateau concentration that can be maintained by constant infusion at a rate of \( I_m \). In this formulation, \( I_m \) is insignificantly altered from the original infusion rate \( I \) (\( I_m = 0.981 \) by derivation).
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K A Fox, A K Robison, R M Knabb, T L Rosamond, B E Sobel and S R Bergmann

Circulation. 1985;72:1346-1354
doi: 10.1161/01.CIR.72.6.1346

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/72/6/1346

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