Pharmacodynamics of tissue-type plasminogen activator characterized by computer-assisted simulation*

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ABSTRACT Prospective characterization of pharmacodynamics of tissue-type plasminogen activator (t-PA) is needed for diverse clinical applications. Accordingly, we used physiologically based, computer simulation of participating biochemical reactions in response to concentrations of circulating t-PA seen with infusions of 1 to 7 hr duration in 45 patients. Predicted values were compared with those from a "training set" obtained in six patients given t-PA for coronary thrombosis and six receiving therapy for peripheral arterial occlusion. Subsequently, results of simulation were compared prospectively with observations from a "test set" of 33 consecutive patients given low doses of t-PA for as long as 7 hr or higher doses for 1 to 2 hr and with data from 101 patients given t-PA in the European Cooperative Trial. Fits between observed and predicted values were close. Based on observations in the training set, the $\alpha_2$-macroglobulin reaction with circulating plasmin and ongoing synthesis of plasminogen were incorporated in the simulations. Fibrinogenolysis in vitro was documented despite supplementation of samples with aprotinin, particularly when concentrations of t-PA were high. This phenomenon can lead to overestimation of fibrinogen depletion and was found to be obviated by the use of PPACK, a novel serine protease inhibitor. Results indicate that the simulation approach developed permits economic, prospective evaluation of regimens of t-PA suitable for diverse conditions and delineation of the impact of individual constituents and reactions on pharmacodynamics of t-PA and on the risk of induction of a systemic lytic state.

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THE PROMISE of coronary thrombolysis was recognized initially in the late 1950s.\(^1\) Its relatively slow acceptance reflected in part the lack of definitive end points of efficacy and risks of associated invasive procedures, bleeding, or allergic reactions with conventional fibrinolytic agents. However, Rentrop's documentation of coronary recanalization with streptokinase in 1979 intensified interest.\(^2\) Recently, decreased mortality with\(^3\)\(^4\) and potential advantages of clot-selective agents were demonstrated.\(^5\)\(^6\) Activation of plasminogen in the systemic circulation gives rise to fibrinogenolysis and elevation of fibrinogen degrada- tion products (FDPs) that exhibit potent anticoagulant activities. In contrast to streptokinase and urokinase,\(^7\) pharmacologically active doses of tissue-type plasminogen activator (t-PA) elicit clot lysis without necessarily activating circulating plasminogen. Thus, its risk/benefit ratio may be superior to conventional, first-generation agents.\(^8\)\(^9\)\(^10\)

Because of the importance of very early intervention for salvage of myocardium,\(^12\)\(^13\) increasingly higher initial doses of t-PA have been used. Regimens being tested presently use doses 10-fold or more greater than those used in the initial study demonstrating coronary thrombolysis with t-PA.\(^5\) However, safe dosage of t-PA is undoubtedly limited by an upper bound.\(^14\)

Benefits of coronary thrombolysis may be nullified by early reclosure of initially recanalized vessels. Antiplatelet drugs and heparin\(^15\)\(^16\) do not appear to prevent this untoward complication. Judging from results in animals, subthrombolytic doses of t-PA can prevent reclosure.\(^17\) However, it is not yet clear to
what extent prolonged administration of such low doses may activate circulating plasminogen.

Prolonged infusions of clot-selective activators may be desirable for treatment of entities other than myocardial infarction, such as pulmonary emboli, deep venous thrombosis, or occlusive peripheral arterial disease. Thus, characterization of the pharmacodynamics of diverse regimens is needed.

Accordingly, we used a physiologically based model and computer simulations to elucidate safe and effective dose regimens and to clarify the relative influence of specific components of the fibrinolytic system on pharmacodynamics of t-PA given in selected doses over a wide variety of intervals.

Simulations employed forward and reverse rate constants for eight constituent reactions of the fibrinolytic system. Predicted and observed values were compared in a “training set” of six patients treated with t-PA for acute myocardial infarction and six patients treated for iliofemoral arterial disease. Subsequently, results obtained with the model were compared prospectively with those obtained in 33 consecutive patients (a “test set”) treated for peripheral arterial disease with prolonged infusions of low doses of t-PA or treated for acute myocardial infarction with shorter infusions of higher doses of t-PA. In addition, results of simulations were compared with data from the literature from 101 patients treated with t-PA in a multicenter cooperative trial.

During the course of these studies, it became clear that fits between predicted and observed values were improved when data were acquired from samples protected adequately against activation of plasminogen in vitro that might otherwise be encountered as a result of freezing and thawing or inadequate inhibition of t-PA in the sample despite conventional supplementation with aprotinin. Such sampling artifact, if not precluded, may distort results of clinical studies of thrombolysis.

Table 1

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Initial concentration in plasma (μM)</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>10</td>
<td>24, 25</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Plasmin</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>(\alpha_1)-Antiplasmin</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>(\alpha_2)-Macroglobulin</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>FDPs</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>Plasminogen fragments</td>
<td>0</td>
<td>27</td>
</tr>
</tbody>
</table>

During the course of these studies, it became clear that fits between predicted and observed values were improved when data were acquired from samples protected adequately against activation of plasminogen in vitro that might otherwise be encountered as a result of freezing and thawing or inadequate inhibition of t-PA in the sample despite conventional supplementation with aprotinin. Such sampling artifact, if not precluded, may distort results of clinical studies of thrombolysis.

Methods

Computer simulation of the pharmacodynamics of t-PA. Selected biochemical reactions occurring in the systemic circulation after administration of t-PA are summarized in table 1. Those included in the computer simulations are numbered. Reactions such as those involving t-PA with circulating inhibitors or inactivators are not included in the simulations because of the relatively low concentrations of the inhibitors with respect to pharmacologic concentrations of t-PA in plasma. Reactions depicting synthesis of fibrinogen, \(\alpha_2\)-antiplasmin, and plasminogen from hypothetical precursor pools are dummy reactions selected to conform to the observed rate of resynthesis of each moiety after cessation of infusion of t-PA in patients in the training set. Because of the relatively slow rate of resynthesis of fibrinogen and \(\alpha_2\)-antiplasmin, the only reaction of this kind incorporated was the one pertinent to plasminogen.

Initial plasma concentrations of moieties referred to in table 1 are shown in table 2. Rate constants, designated as “k” values, are listed in table 3. Subscripts correspond to the reaction numbers delineated in table 1: forward rate constants are shown with positive subscripts and reverse rate constants are shown with negative subscripts. For irreversible reactions, reverse rate constants are set at zero.

The concentration of t-PA in plasma for treated patients was calculated from the dose–plasma concentration relationship.20 Directly measured values conformed quite closely to calculated values (r = .97) and to estimates obtained by conventional pharmacokinetic analysis (r = .98) with the use of a one-compartment model and a distribution volume for t-PA equal to plasma volume. Thus, even though plasma t-PA clearance in animals and patients comprises a second (β) exponential component* compatible with distribution of t-PA into more than one compartment or with late release from tissue, its influence is

TABLE 3
Rate constants of reactions accompanying infusion of t-PA

<table>
<thead>
<tr>
<th>Constant</th>
<th>Value</th>
<th>Reference No.</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_0 )</td>
<td>0.3 sec(^{-1} )</td>
<td>24</td>
<td>Calculated based on measured ( K_m ) and the assumption that the</td>
</tr>
<tr>
<td>( k_{-1} )</td>
<td>280 sec(^{-1} )</td>
<td>24</td>
<td>rate is diffusion limited</td>
</tr>
<tr>
<td>( k_1 )</td>
<td>10 ( \mu )M(^{-1} ) sec(^{-1} )</td>
<td>24</td>
<td>Based on an apparent ( K_m ) with fibrinogen present and the</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>30 ( \mu )M(^{-1} ) sec(^{-1} )</td>
<td>25, 26</td>
<td>calculated value for ( k_1 )</td>
</tr>
<tr>
<td>( k_3 )</td>
<td>0.0063 sec(^{-1} )</td>
<td>25, 26</td>
<td>Reaction assumed to be irreversible</td>
</tr>
<tr>
<td>( k_4 )</td>
<td>0.004 sec(^{-1} )</td>
<td>25</td>
<td>Calculated based on measured ( K_m )</td>
</tr>
<tr>
<td>( k_5 )</td>
<td>10 ( \mu )M(^{-1} ) sec(^{-1} )</td>
<td>27</td>
<td>Reaction assumed to be irreversible</td>
</tr>
<tr>
<td>( k_6 )</td>
<td>300 sec(^{-1} )</td>
<td>27</td>
<td>Estimated from the first-order rate constant for cleavage</td>
</tr>
<tr>
<td>( k_7 )</td>
<td>25 sec(^{-1} )</td>
<td>28</td>
<td>of synthetic substrate</td>
</tr>
<tr>
<td>( k_8 )</td>
<td>1.85 \times 10^{-7}</td>
<td>25</td>
<td>Reaction assumed to be irreversible</td>
</tr>
<tr>
<td>( k_9 )</td>
<td>4.6 \times 10^{-6}</td>
<td>—</td>
<td>Estimated by analogy with measured interactions of ( \alpha_2 )</td>
</tr>
<tr>
<td>( k_{-9} )</td>
<td>—</td>
<td>—</td>
<td>macroglobulin with several plasminogen</td>
</tr>
<tr>
<td>( k_{-7} )</td>
<td>—</td>
<td>25</td>
<td>Reaction assumed to be irreversible</td>
</tr>
<tr>
<td>( k_+ )</td>
<td>0.35</td>
<td>29, 30</td>
<td>Based on the observed rate of increase of concentration of</td>
</tr>
<tr>
<td>( k_{-5} )</td>
<td>1.85 \times 10^{-7}</td>
<td>—</td>
<td>plasma plasminogen after cessation of infusion of t-PA</td>
</tr>
</tbody>
</table>

Subscripts refer to reactions as numbered in table 1. Positive and negative subscripts refer to forward and reverse reactions, respectively.

quantitatively modest in patients given infusions of t-PA over intervals as long as 7 hr.

Simulations were performed with a Vax 11/780 computer with software (KINSIM\(^2\)) kindly provided by Dr. Carl Freiden. KINSIM permits compilation of a series of as many as 40 chemical reactions, each of which can be represented by a standard format equation entailing forward and reverse rate constants or rapid equilibrium. Parameters set by the operator include initial (or constant) concentrations of constituent components, values for each of the rate and equilibrium constants, intervals during which the reactions proceed, and scale factors for selected output values. The program provides a continuous solution for the interactive differential chemical equations by numerical integration using derivatives at each selected time point to estimate concentrations of each component. Display options include lists of output values, CRT displays, and hard copy plots of selected output.

Initial simulations were found to overestimate the extent of depletion of fibrinogen and the rate of decline of \( \alpha_2 \)-antiplasmin. These initial simulations were performed without inclusion of a reaction involving \( \alpha_2 \)-macroglobulin because of our assumption that the rapid interaction between plasmin and \( \alpha_2 \)-antiplasmin would obviate the importance of the relatively slower interaction between plasmin in the circulation and \( \alpha_2 \)-macroglobulin. As illustrated in figure 1, however, the simulation is quite sensitive to the presence or absence of a component reaction involving \( \alpha_2 \)-macroglobulin. After prolonged infusions of t-PA, consumption of \( \alpha_2 \)-antiplasmin is considerable. Under these conditions, any plasmin released into the circulation will induce fibrinogenolysis (figure 1, A). In contrast, in the presence of circulating \( \alpha_2 \)-macroglobulin plasmin will be neutralized, albeit more slowly than would be the case if \( \alpha_2 \)-antiplasmin were still present in substantial concentrations. Because the observed values in our training set patients conformed so much more closely to the pattern indicated in figure 1, B, subsequent simulations included the \( \alpha_2 \)-macroglobulin reaction.

Another pertinent factor was identified in the training set studies. Although the rate of resynthesis of fibrinogen was quite slow (with 50% or less of depleted fibrinogen resynthesized within 24 hr judging from our observations), resynthesis of plasminogen was rapid. In our training set of treated patients given prolonged infusions of t-PA, the increase in the concentration of plasminogen within 12 hr after cessation of the infusion of t-PA was equivalent to 40% of the baseline, zero-time concentration. Thus, a dummy reaction was incorporated in the simulations with forward and reverse rate constant values selected such that resynthesis would be consistent with the changes in concentration after marked depletion and such that steady-state concentrations would prevail under physiologic conditions. The absolute values of the constants used are of course dependent on the arbitrarily selected value of the precursor pool size, which we designated at 50 \( \mu \)M. When this reaction was not included in the simulations, observed values for plasminogen were substantially higher than those predicted.

**Patients studied.** Data were obtained from patients given t-PA by infusion over 1 to 7 hr in total doses ranging from 0.05 to 0.5 mg/kg/hr. Twenty-five patients were studied at The Cleveland Clinic. Their ages ranged from 28 to 76 years. Each had angiographically documented peripheral arterial thrombotic occlusion, generally in the iliofemoral system, or thrombotic occlusion of a peripheral arterial bypass graft. Each was treated with a regional arterial infusion of 0.05 to 0.1 mg/kg/hr of recombinant DNA-produced t-PA (rt-PA) for 1 to 7 hr. Twenty patients were studied at Washington University. Their ages...
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FIGURE 1. Simulations of serial changes in plasma constituents designated during infusion of t-PA sufficient to maintain the concentration of plasma t-PA at 10 nM for 2 hr. A, Results under conditions in which reactions of plasmin with α2-macroglobulin are neglected. Once α2-antiplasmin has been consumed, profound fibrinogenolysis ensues. FDPs are expressed as percentages of baseline fibrinogen. B, Results under conditions in which the interaction between plasmin and α2-macroglobulin is considered. A modest reduction in the rate of consumption of α2-antiplasmin occurs compared with that in A because of the interaction of plasmin with α2-macroglobulin. However, even when consumption of α2-antiplasmin is almost complete, marked fibrinogenolysis does not occur because of the interaction of plasmin being formed with α2-macroglobulin.

ranged from 38 to 74 years. Each was treated with rt-PA by an intravenous infusion of 0.5 mg/kg/hr for 1.5 hr or 0.375 mg/kg/hr for 2 hr.

The regimens used were effective. Thus, in patients with peripheral vascular disease who were given low doses of t-PA for intervals as long as 7 hr, recanalization was documented angiographically in every case. Among the patients with evolving myocardial infarction who were treated with higher doses over briefer intervals, recanalization was documented angiographically in 75%, an efficacy rate equivalent to that reported in previous studies.

Six patients with peripheral arterial occlusion and six with evolving myocardial infarction were studied initially and comprised a training set with which the computer-assisted simulation procedure was developed. The additional 33 patients comprised a test set for the simulation procedure. In addition, results from the literature from 101 patients evaluated in the European Cooperative Trial of t-PA for coronary thrombolysis were compared with values predicted by the computer simulation procedure. The recanalization rate reported (74%) was comparable to that observed in our patients and to results of previous studies.

Biochemical methods. rt-PA was supplied by Genentech, Inc., South San Francisco, and assayed radioimmunometrically and functionally with the use of a fibrin plate assay; plasminogen and α2-antiplasmin were assayed amidolytically, fibrinogen was assayed with the sodium sulfite precipitation method, and FDPs were assayed conventionally as described previously. In brief, a two-site immunoradiometric assay was used for detection and quantification of t-PA antigen in frozen plasma samples collected with citrate, thawed, diluted serially, and subjected to duplicate analyses at each dilution. Results were calibrated with a standard curve obtained with purified human melanoma cell-derived t-PA. This material and rabbit antibody to human t-PA were kindly provided by Professor Désiré Collen.

Functional t-PA activity was determined with fibrin plates exposed to euglobulin fractions prepared by 1:20 dilutions of citrated plasma with distilled water, adjustment of pH to 5.8 with acetic acid, and centrifugation. After suspension of precipitates in a volume equal to the original volume of citrated plasma in buffer containing 0.08% bovine serum albumin, duplicate spots were applied on fibrin plates prepared with fibrinogen from Kabi Diagnostica. Quantification of zones of lysis by planimetry was performed after plates had been incubated at 37°C for 16 hr and calibrated with reference to the international reference standard of t-PA.

Results of the amidolytic procedure used for assay of plasminogen and α2-antiplasmin were expressed as percentages of normal values in pooled human plasma. Reduction of amidolysis otherwise induced with exogenous plasmin (Kabi Diagnostica) reflected α2-antiplasmin present in the sample. Results were expressed as percentages of α2-antiplasmin activity in normal, pooled human plasma.

For assays of fibrinogen by the sodium sulfite precipitation method, plasma samples were collected in Vacutainer tubes. Determinations were made in freshly obtained samples or in samples frozen and thawed only once.

Data from the European Cooperative Trial were obtained with analogous procedures.

Acquisition and protection of samples. Peripheral venous blood samples were acquired with a two-syringe technique, avoiding contamination of samples with contents of the dead space of indwelling venous catheters. Blood samples were placed immediately on ice and centrifuged at 4°C. Separated plasma was either assayed immediately or frozen at −70°C before assay. Samples used for determination of fibrinogen were collected in citrate supplemented with aprotinin (200 KIU/ml blood) to inhibit fibrinogenolysis in vitro. Those used for determination of α2-antiplasmin or plasmin were not exposed to aprotinin.

In most studies of activators of the fibrinolytic system aprotinin is added to samples in concentrations in the range of 100 to 500 KIU/ml blood to avoid formation of plasmin in vitro by activator present in the sample. Such additions may interfere with assays of α2-antiplasmin or plasminogen because they may inhibit serine protease–mediated formation of plasmin in the assay system. On the other hand, they are deemed essential for accurate determination of fibrinogen that might otherwise be depleted in vitro. Judging from our own and Professor Collen’s experience, it became clear that conventional inhibition of exogenous t-PA in samples from treated patients is often insufficient for prevention of fibrinogenolysis in vitro. Failure is particularly striking in samples acquired when circulating plasma concentrations of t-PA are very high in patients treated with...
large doses. Accordingly, in close collaboration with Dr. Adair Hotchkiss and his co-workers at Genentech, Inc., who were characterizing alternative means for prevention of artifactual formation of plasmin in vitro, we evaluated another inhibitor of serine proteases, o-phenylalanyl-l-prolyl-l-arginine chloromethyl ketone·2HCl (PPACK), for inhibition of t-PA in samples from patients given large doses of t-PA for treatment of coronary thrombosis as follows.

PPACK (acquired from Calbiochem) was prepared as a 400 μM stock solution in 0.01N HCl and stored frozen. Blood samples were drawn into EDTA-Vacutainer tubes containing 74 μl of 1N HCl per 2 ml of blood (to lower the pH to 4.2 to avoid lability of the PPACK added subsequently). Ten microliters of stock solution of PPACK was added to tubes in which 2 ml samples were to be obtained, and 25 μl was added to tubes in which 5 ml samples of blood were to be obtained. Tubes were frozen on dry ice in acetone. The contents were lyophilized to dryness before storage in a dessicator with silica gel at 0° to 4° C. Blood samples were collected by syringe and transferred as rapidly as possible to the previously prepared Vacutainer tubes, thoroughly mixed by inversion, cooled to 0° to 4° C, and maintained on ice for 1 hr. Plasma was separated by centrifugation and frozen at -20° C for at least 1 hr. Samples were thawed before assay. Under these conditions, PPACK neutralized t-PA in the sample, but because of its lability under the conditions of freezing and thawing, all but 10% or less of the PPACK that had not combined with t-PA had degraded spontaneously by the time samples were assayed, as shown by thrombin time assays performed with serial dilutions.

PPACK did not alter results in samples without exogenous t-PA. In samples from patients given t-PA, PPACK prevented spurious diminution of fibrinogen and plasminogen and artifactual, apparent consumption of α2-antiplasmin.

Results

Comparison of observed with predicted values. Figure 2 illustrates the concordance between observed and predicted values for plasma fibrinogen and plasminogen in samples from a typical patient in the test set given 25 mg of rt-PA over a 7 hr interval. The observed activation of plasminogen reflected by its consumption was paralleled by a more modest diminution of circulating fibrinogen. Values predicted by the computer simulation conformed closely to those observed in serial samples obtained throughout the infusion of rt-PA.

Results shown in figure 3 are average values ± SDs of measured concentrations of plasminogen in all 44 plasma samples collected over intervals of 1 to 7 hr after the onset of infusion of t-PA in patients in the test set. Because the dose of t-PA was varied purposefully over a wide range and because samples were obtained at specified intervals throughout the infusions, absolute magnitudes of diminution of plasminogen with respect to values in zero-time samples varied widely. As can be seen, however, observed values correlated with those for all four quartiles of predicted values.

When the computer simulation was applied to blood levels of t-PA and durations of infusion prevailing in the European Cooperative Trial, the predicted changes in concentrations of fibrinogen and plasminogen throughout the 90 min infusions of t-PA were similar to observed values. As illustrated in figure 4, results from 101 patients given 60 mg of t-PA over 90 min correlated closely with those predicted by the computer simulation. These results are consistent with those obtained in samples from our test set of patients and
support the view that the computer simulation provides reasonably good estimates of changes of fibrinogen and plasminogen as a function of duration and dose of infusion of t-PA.

Identification of strong determinants of pharmacodynamics. When the computer simulation was used to explore the potential effects of t-PA infusions of different doses and durations, several salient phenomena were evident. As illustrated in figure 5, A, although the magnitude of depletion of plasminogen anticipated is substantial under conditions in which a concentration in plasma of 10 nM t-PA is assumed to prevail throughout a 1 hr infusion, marked systemic fibrinogenolysis is not predicted. Thus, the computer simulation predicts a decline of circulating plasminogen from approximately 2 to 1.5 μM when plasma t-PA is maintained at a concentration of 10 nM throughout the duration of the infusion. In marked contrast, however, a comparable concentration of t-PA maintained for 4 hr is predicted to result in a more marked diminution of plasminogen to a concentration of approximately 0.5 μM at the end of the infusion — a change consistent with induction of considerable fibrinogenolysis. Ten nanomoles t-PA is equivalent to approximately 700 ng/ml, i.e., a very modest, “therapeutic” level. Accordingly, the computer simulation portends substantial systemic fibrinogenolysis despite the maintenance of a concentration of t-PA in plasma very near the lower limit of the therapeutic range throughout a moderately long infusion.

Analogous observations were obtained by application of the computer simulation to estimation of changes in plasma fibrinogen as a function of the pre-

FIGURE 4. Simulated vs observed values for plasma fibrinogen and plasminogen (expressed as percentages of values at baseline) in samples from 101 patients from the European Cooperative Trial who were treated for coronary thrombolysis with t-PA by continuous intravenous infusion for 90 min in doses anticipated to yield a prevailing plasma level of 20 nM.¹⁹

FIGURE 5. A, Simulated plasma concentrations of plasminogen (plotted on the ordinate) as a function of prevailing concentration of t-PA over intervals of 1 or 4 hr infusions. t-PA values on the abscissa are plotted on a logarithmic scale. The simulation indicates marked diminution of plasma plasminogen at the end of a 4 hr infusion even when plasma concentrations of t-PA do not exceed 10 nM. On the other hand, anticipated diminution of plasminogen is only modest at the end of a 1 hr infusion with a plasma concentration of this magnitude. Conversely, even a brief infusion of 1 hr is anticipated to lead to marked depletion of plasma plasminogen when the prevailing concentration of t-PA approaches 100 nM. B, Plasma fibrinogen (percentage of control) simulated as a function of the prevailing concentration of plasma t-PA throughout a 1 hr infusion. Plasma t-PA concentrations on the abscissa are on a logarithmic scale. Simulated fibrinogen depletion reaches a limiting value despite hypothetically increasing concentrations of plasma t-PA throughout the 1 hr infusions. This phenomenon reflects the consumption of plasminogen and hence the paradoxical lack of generation of plasmin in the circulation when maintained concentrations of t-PA in plasma approach 100 nM.
vailing concentration of t-PA in plasma throughout 1 hr infusions. As shown in figure 5, B, the estimated magnitude of depletion of fibrinogen at the end of such infusions is trivial when plasma t-PA concentrations are assumed to be in the low therapeutic range (approximately 10 nM). However, with concentrations of t-PA approaching 100 nM (equivalent to 7000 ng/ml, i.e., within in the mid to high therapeutic range in recent studies\textsuperscript{6, 8, 9, 23}), the concentration of fibrinogen at the end of the infusion is anticipated to be reduced by approximately 30\% in comparison with control values. These estimates are compatible with the view that the clot selectivity of t-PA is a relative rather than an absolute property.\textsuperscript{6, 9, 20}

Protection of samples. In our initial computer simulations, it was apparent that observed depletions of fibrinogen and plasminogen were more substantial than those predicted by the physiologically based model, especially in samples with high concentrations of t-PA. Furthermore, the variation of plasma levels of fibrinogen despite consistent doses of t-PA in some studies\textsuperscript{8, 9, 23} led us to consider the possibility that degradation of fibrinogen in vitro might have distorted results. Accordingly, we examined the possibility that artificial generation of plasmin in vitro and consequent fibrinogenolysis in samples subjected to freezing and thawing may have confounded results despite the inclusion of aprotinin in the samples. One set of samples from patients given large amounts of t-PA (i.e., those treated for coronary thrombosis) was supplemented with PPACK. Another was supplemented conventionally with aprotinin. In control studies, we found that PPACK did not detectably alter fibrinogen values with or without added t-PA in concentrations of from 100 to 10,000 ng/ml of plasma and repeated freezing and thawing. Analogous consistency of assays of $\alpha_2$-antiplasmin was evident in samples supplemented with 2 $\mu$M PPACK and treated similarly.

Supplementation of samples with PPACK led to preservation of fibrinogen in comparison with results in samples treated with aprotinin or the citrate anticoagulant, as shown in figure 6. Thus, in samples protected with PPACK from a patient given 60 mg of t-PA over 1 hr followed by 40 mg over 2 hr assayable fibrinogen was as much as threefold higher compared with values in samples protected only with aprotinin or in those unprotected with any serine protease inhibitor. In general, the largest disparities were evident in samples from patients in which the concentration of t-PA was maximal.

As illustrated in figure 7, the concordance between measured changes in fibrinogen and those predicted by computer simulation was particularly close when measured values were those obtained in samples protected with PPACK. In contrast, apparent values for fibrinogen in samples from the same patient given 60 mg of t-PA over 1 hr were substantially lower when samples were protected conventionally with aprotinin but not PPACK. Because PPACK does not affect the fibrinogen assay, the lower fibrinogen values in samples without PPACK from patients with high blood levels of t-PA appear to reflect fibrinogenolysis in vitro.
Discussion

Results in this study indicate that physiologically based computer simulation permits accurate estimation of pharmacodynamic effects of t-PA on the fibrinolytic system in circulating blood under a wide variety of conditions. Thus, the approach appears likely to be useful for defining boundaries for dose regimens potentially valuable for the treatment of diverse conditions such as pulmonary emboli, ventricular mural thrombi, deep venous thrombosis, cerebral vascular disease, and coronary thrombosis and for prevention of coronary reocclusion. Results also indicate that adequate protection of plasma samples with serine protease inhibitors is needed to avoid fibrinogenolysis in vitro that can distort interpretation.

The computer simulations indicate that pharmacodynamic effects of t-PA are markedly dependent on several factors. The initial concentration of α2-macroglobulin and the participation of α2-macroglobulin as an inhibitor and neutralizer of plasmin are important, despite the fact that the association of plasmin with α2-antiplasmin is much more rapid than that of plasmin with α2-macroglobulin. De novo synthesis of plasminogen is an important determinant of prevailing concentrations, especially in patients treated with prolonged infusions of t-PA.

Pharmacodynamic effects of t-PA on constituents of the fibrinolytic system in the circulating blood are dependent on not only the absolute magnitude of the dose of t-PA but also the duration of infusion. Under circumstances in which α2-antiplasmin is depleted, a systemic lytic state is precluded by the interaction of α2-macroglobulin with plasmin. It is apparent that depletion of α2-macroglobulin would predispose to a systemic lytic state as a result of any further elaboration of plasmin in the circulation.

Marked consumption of plasminogen may result in paradoxical phenomena. Administration of additional activator under such circumstances would not lead to formation of plasmin in the circulation. Thus, further diminution of circulating fibrinogen would not occur. As shown in figure 5, simulated sequential values of fibrinogen approach an apparent limit with prolonged, prevailing concentrations of t-PA in plasma approaching 100 nM.

In view of the unavoidably large expense of studies of newly developed agents such as rt-PA and the considerable cost of large-scale clinical trials, economic estimates of pharmacodynamic effects of t-PA and other promising activators of the fibrinolytic system should be useful. Results of this study indicate that computer simulation of reactions consequent to exposure of circulating plasminogen to pharmacologic concentrations of t-PA with incorporation of experimentally determined forward and reverse rate constants for individual reactions and prevailing concentrations of components of the system at zero time provide reasonable estimates of pharmacodynamic responses of the system under diverse conditions. Results with PPACK appear to explain, at least in part, the wide intrastudy variation in some reports of apparent fibrinogen depletion despite consistent dose regimens of t-PA. The approach developed provides a convenient and economic means for assessing the relative sensitivity of pharmacodynamics of t-PA to changes in concentrations of specific constituents of the fibrinolytic system in circulating blood, to specific choices of individual rate constants, and to rates of synthesis of components of the fibrinolytic system in vivo. Thus, it should prove useful for defining dose regimens required for specific clinical indications, for elucidating risk/benefit ratios that can be tested in highly focused trials involving relatively small numbers of patients, and for identifying potentially attractive therapeutic strategies with t-PA alone or in combination with other agents.

Addendum

After this manuscript had been initially reviewed, a report from the first phase of the NHLBI Thrombolysis in Myocardial Infarction (TIMI) trial appeared (Williams DO et al: Intravenous recombinant tissue type plasminogen activator in acute myocardial infarction: a report from the NHLBI Thrombolysis in Myocardial Infarction (TIMI) trial. Circulation 73: 338, 1986). Patients described were treated with 40 mg of t-PA intravenously over 1 hr followed by 20 mg/hr for 2 hr. At the end of the 3 hr infusion, fibrinogen values were 70 ± 16% of baseline (n = 32). These observed values agreed within 2% with those predicted by the simulation procedure developed in this study.

References

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