Thrombolysis, clot selectivity, and kinetics

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WHEN the editorial offices of Circulation moved to St. Louis, a series of "Perspectives" was initiated. The first, published in August 1983, dealt with coronary thrombolysis. It presaged the publication in Circulation of 11 manuscripts concerning thrombolysis within the next 12 months. An additional 13 are presently in review. One focus of consideration has been the potential importance of "clot selectivity" of activators of fibrinolysis such as tissue-type plasminogen activator (t-PA).

Advantages of clot selectivity. Clot selectivity appears to render t-PA useful for coronary thrombolysis in part because:

1. Clot lysis can be accomplished with t-PA in doses that do not induce a systemic lytic state characterized by consumption of α2-antiplasmin, depletion of plasminogen, fibrinogenolysis, increased circulating fibrinogen degradation products (FDPs), and predisposition to systemic bleeding.

2. Definitive surgical treatment of high-grade residual coronary stenosis can be initiated promptly after successful thrombolysis with t-PA in contrast to conventional activators because of its short biological half-life (approximately 5 min) and its lack of induction of a systemic lytic state.

3. Bleeding from arteriotomy sites may be less frequent after lysis induced with t-PA compared with streptokinase and other non-clot selective activators, perhaps because of the conspicuous, anticoagulant effects of elevated FDPs and prolonged depletion of fibrinogen.

4. Early treatment of suspected infarction offering the greatest opportunity for myocardial salvage may be more justifiable with a clot-selective activator than with streptokinase or urokinase because risks associated with a systemic lytic state can be avoided; and

5. Systemic administration of large doses may be more practical with t-PA compared with streptokinase or urokinase without the need for intensive monitoring of the fibrinolytic system because of its clot selectivity.

The efficacy of coronary thrombolysis. The ultimate place of coronary thrombolysis in the therapy of acute myocardial infarction depends on resolution of several questions, including:

1. The frequency of early and late coronary reocclusion after initially successful thrombolysis;

2. The extent to which successful thrombolysis can salvage myocardium;

3. The extent to which coronary thrombolysis can preserve ventricular function;

4. The value of delay of necrosis by thrombolysis in providing time for coronary artery bypass grafting or angioplasty;

5. The incidence and clinical implications of arrhythmogenesis early and late after initially successful coronary thrombolysis; and

6. Long-term effects on morbidity and mortality of coronary thrombolysis alone, in combination with drugs designed to potentiate myocardial salvage, or followed by surgery or angioplasty.

The nature of clot selectivity. This discussion will focus primarily on the nature of clot selectivity of activators of the fibrinolytic system such as t-PA. We wish not only to underscore the potential advantages of this property but also to circumscribe the meaning of the term "clot selectivity," emphasizing its relative rather than absolute nature. We believe that such circumscription is necessary to preclude unrealistic expectations regarding t-PA and other clot-selective activators. Consideration of the kinetics of the fibrinolytic system leads to the following obvious though to date perhaps underemphasized conclusion. The safety of t-PA, and for that matter the safety of any agent — even water — is a relative, dose-dependent property, not an absolute characteristic.

Reactions involved in fibrinolysis. Physiologic clot lysis results from several reactions, including:

1. Hydrophobic binding of t-PA to fibrin and noncovalent binding of plasminogen to fibrin through lysine binding sites on the plasminogen molecule; and

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Activation of fibrin-bound plasminogen by fibrin-bound t-PA to form plasmin juxtaposed to fibrin and relatively free from the neutralizing effect of circulating \( \alpha_2 \)-antiplasmin.

Plasmin formed at the fibrin surface is a serine protease capable of rapidly inducing clot lysis. Any plasmin escaping into the circulation is rapidly inactivated by \( \alpha_2 \)-antiplasmin, present in considerable excess. Thus, under physiologic conditions circulating \( \alpha_2 \)-antiplasmin is not appreciably consumed, circulating fibrinogen is not degraded, and circulating FDPs do not increase. In contrast, fibrinolysis induced pharmacologically with streptokinase or urokinase results in conversion of circulating plasminogen to plasmin; consumption of circulating \( \alpha_2 \)-antiplasmin; degradation of circulating fibrinogen, plasminogen, and other plasma proteins by plasmin; augmentation of circulating FDPs; and impairment of systemic hemostasis.

Fibrinolysis induced pharmacologically with t-PA resembles physiologically induced fibrinolysis much more than it resembles fibrinolysis induced pharmacologically with streptokinase or urokinase. The affinity of free, circulating t-PA for circulating plasminogen is low in contrast to the affinity of t-PA for plasminogen when both are bound to fibrin in a thrombus. Thus plasmin does not accumulate in circulating plasma but is formed at the fibrin surface of the clot.

Reactions that may occur when t-PA is administered pharmacologically include the following:

\[
\begin{align*}
(1) & \quad t-PA + P_g \rightleftharpoons [t-PA \cdot P_g] \rightarrow \text{plasmin} + P_g \text{ fragment} + t-PA \\
(2) & \quad \text{plasmin} + \alpha_2 \text{AP} \rightleftharpoons [\text{plasmin} \cdot \alpha_2 \text{AP}] \rightarrow \text{inactive product} \\
(3) & \quad \text{plasmin} + F_g \rightleftharpoons [\text{plasmin} \cdot F_g] \rightarrow \text{plasmin} + \text{FDPs}
\end{align*}
\]

where \( P_g \) = plasminogen, \( \alpha_2 \text{AP} = \alpha_2 \)-antiplasmin, \( F_g \) = fibrinogen, and brackets [ ] refer to complexes of two constituents; \( k_1 \), \( k_2 \), \( k_3 \), \( k_4 \), \( k_5 \), and \( k_6 \) represent the forward rate constants and \( k_{-1} \), \( k_{-3} \), and \( k_{-5} \) the reverse reaction rate constants for those reactions considered to be reversible. For any pair of rate constants with the same numerical subscript but opposite sign (e.g., \( k_1 \) and \( k_{-1} \)) the inverse ratio of the two is equal to the apparent Michaelis constant \( (K_m) \) for the reaction. The close spatial association of t-PA and plasminogen on a fibrin surface facilitates the interaction of the two moieties. Thus the apparent \( K_m \) for the reaction when both moieties are associated with fibrin is 0.14 \( \mu \text{M} \) compared with 65 \( \mu \text{M} \) when both are free in solution. The ratio of the two apparent \( K_m \) values reflects the striking difference between affinity of t-PA for plasminogen under the two sets of conditions. Accordingly, the anticipated reaction rate is markedly greater when both moieties are associated with fibrin compared with the case when both are free in the circulation. This accounts for the clot selectivity of t-PA.

**Qualitative implications of kinetics.** It is clear from consideration of reactions (1), (2), and (3) that under some conditions associated with pharmacologic administration of t-PA, plasmin could appear in the circulation in quantities sufficient to consume \( \alpha_2 \)-antiplasmin, induce fibrinogenolysis, and increase FDPs. If an infinite amount of t-PA were present in the circulation, reaction (1) would proceed rapidly despite the relatively low affinity of circulating t-PA for circulating plasminogen reflected by the apparent \( K_m \). If the amount of plasmin formed were sufficient to drive reaction (2) to the right, \( \alpha_2 \)-antiplasmin would be consumed, free plasmin would accumulate in the circulation, and a systemic lytic state would ensue. However, the concentration of circulating t-PA is limited by its short biological half-life (approximately 5 min) and by the dose and duration of infusion of t-PA. Accordingly, clot selectivity can be defined in terms of the prevailing plasma concentration of pharmacologically administered t-PA. Clot-selective dosage regimens are those yielding a concentration of t-PA sufficient to lyse clots rapidly but too low to form circulating plasmin in quantities sufficient to allow reaction (3) to proceed.

To delineate upper bounds of dosage that meet these criteria and hence define "clot selectivity" of t-PA quantitatively, it would be necessary to characterize the time-dependent concentrations of constituents of the fibrinolytic system for numerous permutations of dose and duration of infusion. This is not imminently practical. Alternatively, one can estimate the upper bounds with the use of a mathematical model of the kinetics involved, assumed initial concentrations of constituents of the fibrinolytic system, and values for rate constants obtained from published studies of purified systems. The model can be used to simulate effects of selected steady-state concentrations of circulating t-PA. Its predictions, however, provide only crude estimates because both the underlying assumptions and the model may require extensive modifications. Nevertheless, estimates obtained with analogous models have often provided insight regarding the relative influence of individual components on end points in complex systems. To illustrate the relative nature of clot selectivity of t-PA, we used a mathematical model reflecting equations (1), (2), and (3) and incorporating the following assumptions:

1. Prevailing plasma levels of t-PA are defined as either
1 nM (10-fold more than the average value in normal subjects⁴), 50 nM (within the range of values seen with infusions of t-PA in patients⁴), or 100 nM (a value selected to simulate levels that would be encountered only with extremely high doses). In vivo, the absolute magnitude of the steady-state level of t-PA under physiologic conditions may be influenced by inhibitors. However, with doses considered here, effects of inhibitors are likely to be negligible and are disregarded for convenience.

(2) Prevailing concentrations of components of the fibrinolytic system at the onset of an infusion of t-PA are assumed to be those shown in table 1.

(3) The rate constants for reactions (1), (2), and (3) in plasma are assumed to be similar to those determined with purified proteins (table 2) despite the oversimplification entailed.

(4) Other factors, known or as yet unidentified, capable of influencing activation of plasminogen and cleavage of fibrinogen are assumed to exert only minor perturbations under the conditions considered.

(5) For purposes of this analysis, the small amount of plasmin interacting with fibrin in clots can be disregarded.

With these assumptions, we employed the model to predict time-dependent changes induced by selected levels of t-PA in concentrations of constituents of the fibrinolytic system indicative of the presence or absence of a systemic lytic state. Computer simulation was performed with a VAX 11/780 computer with a program, KINSIM, kindly provided by Dr. Carl Frieden.¹⁵

**Quantitative implications.** Results predicted by the model under conditions in which selected levels of t-PA were maintained for 1 hr to simulate a 1 hr infusion are illustrated in figure 1. As can be seen, 10-fold greater than physiologic levels of circulating t-PA are not predicted to consume circulating α₂-antiplasmin, diminish circulating plasminogen or fibrinogen, or appreciably increase FDPs (changes that would be indicative of a systemic lytic state). With 50 nM t-PA maintained for 1 hr, plasminogen and α₂-antiplasmin are predicted to decrease considerably and fibrinogen to decline by 20%. FDPs are predicted to accumulate modestly. With a plasma level of t-PA of 100 nM, approximately 1000-fold greater than the physiologic level and one likely to be encountered only with extremely high doses, α₂-antiplasmin and plasminogen are predicted to decline markedly, and fibrinogen is predicted to decline by 25% within 1 hr. Thus t-PA is not predicted to be clot selective at this dosage.

**Clinical implications.** The implications of a mathematical model of the kinetics involved in t-PA–induced fibrinolysis are not intended to prospectively define dose regimens. They are presented to underscore the relative nature of clot selectivity that can be reasonably anticipated for t-PA and in fact for any activator participating in qualitatively similar biological interactions. The model reflects the fact that large, sustained doses of t-PA are capable of overwhelming the low affinity of t-PA for circulating plasminogen and hence of eliciting systemic fibrinogenolysis. This conclusion does not detract from the potential clinical utility of t-PA as a coronary thrombolytic agent. In fact, the analysis indicates that a wide margin separates toxic from therapeutic regimens because of clot selectivity. Nevertheless, it is theoretically possible with unusually high doses to obviate the clot

### Table 1

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Initial concentration in plasma (μM)</th>
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<tr>
<td>Plasminogen⁴</td>
<td>2</td>
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<tr>
<td>Plasmin⁵</td>
<td>0</td>
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<tr>
<td>Plasminogen inactive fragment⁵</td>
<td>0</td>
</tr>
<tr>
<td>FDPs⁵</td>
<td>0</td>
</tr>
<tr>
<td>α₂-antiplasmin⁴</td>
<td>1</td>
</tr>
<tr>
<td>Fibrinogen⁴</td>
<td>10</td>
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</table>

⁴Reference 16.

⁵Assumed to be zero on the basis of observations in normal subjects.

⁶Reference 17.

### Table 2

<table>
<thead>
<tr>
<th>Rate constant</th>
<th>Value</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>k₁</td>
<td>10 μM⁻¹ sec⁻¹</td>
<td>18 A</td>
<td></td>
</tr>
<tr>
<td>k₋₁</td>
<td>280 sec⁻¹</td>
<td>18 Based on an apparent Kᵢ with fibrinogen present of 28 μM and the value of k₁</td>
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</tr>
<tr>
<td>k₂</td>
<td>0.3 sec⁻¹</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>k₃</td>
<td>10 μM⁻¹ sec⁻¹</td>
<td>16 A</td>
<td></td>
</tr>
<tr>
<td>k₋₃</td>
<td>0.0021 sec⁻¹</td>
<td>16 A</td>
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</tr>
<tr>
<td>k₄</td>
<td>0.004 sec⁻¹</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>k₅</td>
<td>10 μM⁻¹ sec⁻¹</td>
<td>19 B</td>
<td></td>
</tr>
<tr>
<td>k₋₅</td>
<td>300 sec⁻¹</td>
<td>19 B</td>
<td></td>
</tr>
<tr>
<td>k₆</td>
<td>25 sec⁻¹</td>
<td>20 C</td>
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</table>

²Estimated from published values of Kᵢ and the assumption that the rate is diffusion limited.

³Estimated from the apparent Kᵢ for the bovine fibrinogen and plasmin interaction and the assumption that kₑ is limited by the diffusion rate of two proteins in solution.

²Estimated from the first-order rate constant for the cleavage of synthetic substrate.

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selectivity of t-PA and to induce formation of plasmin in the systemic circulation.

As more information is gathered, predictions made with a model such as the one considered here may become sufficiently accurate to provide quantitative guides for dosage of t-PA and other activators of the fibrinolytic system. For the present, such a model may help to formalize therapeutic properties. The potential value of clot selectivity exhibited by t-PA is readily apparent. However, for clot selectivity, as for so much else, all is relative.

References
