Synergism of thrombolytic agents: investigational procedures and clinical potential

D. Collen, M.D., Ph.D.

TWO PHYSIOLOGIC plasminogen activators, tissue-type plasminogen activator (t-PA) and single-chain urokinase-type plasminogen activator (scu-PA, prourokinase), are presently under clinical investigation as fibrin-specific thrombolytic agents. Although recombinant t-PA has been shown to be more effective and clot specific for coronary arterial thrombolysis than streptokinase, its risk/benefit ratio may not be optimal because of only relative fibrin specificity and the occurrence of major bleeding at very high doses. Similar limitations will very probably apply to scu-PA. Methods to enhance the effectiveness of thrombolytic therapy may therefore have significant practical importance. One such approach may be the use of synergistic combinations of thrombolytic agents with different mechanisms of fibrin specificity, such as t-PA and scu-PA.

Synergistic and antagonistic interactions of drugs used for thrombolysis would have significant implications if synergism, as pharmacologically defined, were more marked for the therapeutic than for the toxic effect, or if antagonism were more marked for the toxic than the therapeutic effect. In addition, synergism might reduce the therapeutic dose in man, thus permitting the use of smaller amounts of costly drugs. Optimal exploitation of synergism of thrombolytic agents for clinical use could possibly identify combinations of agents that may result in more optimal risk/benefit and cost/benefit ratios for thrombolytic therapy than currently obtainable.

Synergism, defined as an effect of a combination of agents that is greater than that expected on the basis of their individual dose-effect relationships, is a topic on which great confusion exists in the medical literature. For example, Berenbaum reviewed 56 articles on the topic of immunosuppression dealing with synergism or additiveness and these incorporated 104 claims that particular combinations of immunosuppressive agents were synergic. Only 14 of these claims were valid, with an additional seven probably valid, because most investigators had used fallacious criteria for determining the nature of drug interactions; specifically, they compared the effect of the agents used in combination with the sum of their effects when used alone. Chou and Talalay have also emphasized that the lack of a theoretical basis for the assessment of effects of drug combinations has hampered the rigorous evaluation of drug interactions. A clear delineation of the clinical utility and limitation of synergistic combinations of thrombolytic agents will therefore depend on the use of a rigorous experimental approach.

Methods for demonstrating drug interactions. Several different methods for estimating the effect of additive combinations of drugs have been developed and are in use at present. All of these methods can be shown to be valid in particular sets of circumstances, whereas no single method appears to have totally general applicability. The main methods, which are described in more detail elsewhere, are briefly illustrated below. The individual agents in the combination are denoted by \( x_i \) (\( i = 1, 2, \ldots, n \)), the combination by \( x_{1,2, \ldots, n} \), and the effects of the constituents by \( E(x_i) \).

**Summation method.** The effect of a noninteractive combination is equal to the sum of the effects of its constituents, or

\[
E(x_{1,2, \ldots, n}) = \sum_{i=1}^{n} E(x_i)
\]  

(1)

This assumption is valid only when all the agents in the combination show linear dose-effect relationships. This model is applicable to the thrombolytic effect of t-PA and scu-PA in the rabbit preparation of jugular vein thrombosis.

**Multiplication method.** The effect of the combination is the product of the effects of its constituents, or

\[
E(x_{1,2, \ldots, n}) = \prod_{i=1}^{n} E(x_i)
\]  

(2)
This rule is valid only when all the agents in the combination have simple exponential dose-response curves. This occurs when discrete targets are inactivated by random single hits by discrete quanta of agent such as may occur with ionizing radiations.

**Fractional product method.** The summation of the effect of two inhibitors can be expressed by the product of the fractional activities

\[ E(x_{1,2}) = E(x_1)E(x_2) \]  

(3)

The fractional product equation describes only mutually nonexclusive first-order behavior, i.e., the dose-effect relationships of \( x_1 \) and \( x_2 \) follow Michaelis-Menten hyperbola. When the dose effect is sigmoidal, the fraction product method underestimates the combined effect for mutually nonexclusive drugs and may lead to false claims of synergism.

**The median effect method.** When dose-effect relationships follow a mass-action law, for example:

\[ E(x_i) = \left[ \frac{x_i}{M_i} \right]^m \]  

(4)

where \( E \) is the fractional effect, \( M \) the dose required for the median (50%) effect, and \( m \) is the order of the reaction, generalized equations for the effects of multiple drugs can be derived, representing the effect of exclusive or nonexclusive drugs that obey first or higher order conditions.

A necessary condition for the application of this method is that the median effect plot in its form

\[ \log \frac{E(x_i)}{1 - E(x_i)} = m \log \frac{x_i}{M_i} \]  

(5)

is linear and that the dose-effect curves of the individual drugs in the combination are parallel.

**The isobole method.** If the agents in a combination do not interact in producing the effect of the combination then, irrespective of the type of dose-effect relations, the isobole for that effect satisfies the equation

\[ \sum_{i=1}^{n} \frac{x_i}{X_i} = 1 \]  

(6)

where the \( X_i \)s are the doses of the agents that individually would produce the same magnitude of effect as the combination. The isobole method is valid for mutually exclusive drugs only. Mutually nonexclusive drugs would appear to be slightly synergistic in the isobologram. In addition it is necessary to find proper combinations of drugs that are equieffective with the individual agents.

For the interaction of two mutually exclusive agents, the isobole describing the effect of the combination reduces to

\[ \frac{A}{A_e} + \frac{B}{B_e} = 1 \]  

(7)

where \( A_e \) and \( B_e \) are the equieffective doses (producing the same quantitative effect) of the individual agents, and \( A \) and \( B \) are the doses of the agents in a combination showing the same specified effect. When two agents interact to produce an effect, the expression in equation 7 is less than 1 in the case of synergy and greater than 1 for antagonism.

Chou and Talalay have stressed that the above equation only holds for mutually exclusive drugs. For mutually nonexclusive drugs the following equation was derived:

\[ \frac{A}{A_m} + \frac{B}{B_m} + \frac{A}{A_m} \cdot \frac{B}{B_m} = 1 \]  

(8)

with \( A_m \) and \( B_m \) representing the concentration yielding the median effect. Mutually nonexclusive drugs would appear to be slightly synergistic when analyzed by the isobole method because the third term in equation 8 would be disregarded.

The concepts behind the analysis of drug interactions by the isobole method can be further illustrated as follows. Suppose that two drugs, \( A \) and \( B \), have dose-response curves as shown in figure 1. At unit dose, each produces an effect of 10%, but when combined at this dose, the effect is not 20% but 90%.

**Figure 1.** Dose-effect curves of agents A and B, showing that the nature of drug interaction cannot be determined by adding drug effects. One unit of A or B each produce an effect of 10% and 1 unit of each together produce a 90% effect. This does not indicate synergy because the dose-effect curves show that 2 units of either drug alone also produce an effect of 90%. Adapted from Berenbaum.
Intuitively this might suggest that A and B are synergistic. However, since a 2 unit dose of either drug alone also produces 90% effect the combination clearly is not synergistic. If one would apply the concepts of isoboles, equation 7 would hold for each specified effect of A and B alone or in any combination and one would correctly conclude that there is no interaction between drugs A and B. With regard to fibrinolysis, dose-response curves of t-PA and scu-PA in a human plasma clot lysis system in vitro are clearly of a sigmoidal nature and show a marked threshold phenomenon, especially for scu-PA.\textsuperscript{12, 13}

Calculation of the expected effect of a combination of two drugs by the method described above requires that the range of doses for each agent includes its equieffective dose ($X_i$). In experiments designed to investigate possible synergy or additivity, the dose of each agent in tested combinations must be less than at least one of its doses used alone; otherwise, the sum of the fractions cannot be 1 or less. When agents are tested at two levels only, zero and $X_i$, separately and combined, it is not possible to say whether they show synergy or zero interaction. Most of the preliminary reports on synergism of thrombolytic agents\textsuperscript{13–16} fail in this respect.

**Synergism of thrombolytic agents**

*Risks obtained in systems in vitro.* The phenomenon of synergism of thrombolytic agents was first investigated in system in vitro with use of a human plasma clot immersed in plasma. Some investigators claimed that they observed synergism in vitro,\textsuperscript{13–15} others found no synergism,\textsuperscript{12, 16, 17} while still others claimed to have observed synergism between t-PA and scu-PA in a molar ratio below 1:4, but not in ratios between 4:1 and 1:4.\textsuperscript{16, 19} Yet, the plasma clot lysis system in vitro is relatively straightforward and most investigators have obtained very similar experimental results. The confusion and apparent conflicts appear to be largely due to shortcomings in experimental design and analysis of data, which did not conform to the requirements outlined above.

To date, two groups have reported on synergism in the human plasma clot lysis system in vitro using an experimental approach and data analysis allowing the demonstration of synergism. The results are summarized in table 1. Lijnen et al.\textsuperscript{17} found that a concentration of 0.75 nM t-PA and 5 nM scu-PA were required to produce 25% lysis in 4 hr and that the combination of 0.38 nM t-PA and 2.5 nM scu-PA, representing a molar ratio in the combination of 1/6.5, was equieffective. These authors concluded that t-PA and scu-PA were not synergistic in vitro. A more extensive analysis of the interaction between t-PA and scu-PA was subsequently reported.\textsuperscript{12, 20} Results for an end point corresponding to 50% lysis in 2 hr are also summarized in table 1. In molar ratios of 4:1, 1:1, and 1:4, equieffective total fractional combinations ranged between 0.7 and 0.8, which were statistically not significantly different from 1 (\textit{p} > 0.05). Gurewich and Pannell,\textsuperscript{19} in a study based on a recalculation of partly unpublished data,\textsuperscript{13} found that t-PA and scu-PA, in molar ratios of 1/6 and 1/12, were equipotent in a fractional ratio of 0.7, which was interpreted as marked synergism, whereas in molar ratios between 1/4 and 4/1 the combinations were purely additive (reported as an algebraic fraction of 1 in table 1).

In summary, all available data obtained in a human plasma clot lysis system in vitro indicate that synergism between thrombolytic agents is, at best, marginal. More importantly, the specific thrombolytic activities of t-PA and scu-PA (e.g., the concentration required to produce 50% lysis in a fixed amount of time) differ by a factor 5\textsuperscript{12, 17} to 10,\textsuperscript{1, 3} whereas their thrombolytic potency in vivo (e.g., the dose required to produce coronary artery reperfusion in 1 hr) is comparable.\textsuperscript{2, 3, 21, 22} Clearly, both in terms of thrombolytic potency and the extent of synergism, there is little correlation between the human plasma clot system in vitro and the results in vivo in patients with thromboembolic disease.

*Results in animal preparations.* In a rabbit preparation of jugular vein thrombosis, significant synergism for thrombolysis was observed between t-PA and scu-PA.\textsuperscript{10} Linear dose-response curves were obtained for these agents, a most surprising finding for such a complex biological phenomenon as the pharmacologic dissolution of a blood clot in vivo, but a finding that simplifies the analysis required to demonstrate synergism. Indeed, when dose-response curves are linear, a noninteractive combination of thrombolytic agents will simply display an effect equal to the sum of the effects of its constituents.\textsuperscript{9} The results of an equieffective dose analysis, expressed for the easily identifiable end point of 25% thrombolysis in 4 hr, are summarized in table 1. Equieffective fractional doses of combinations of t-PA and scu-PA were 0.4. Statistical analysis revealed that, compared with additive effects, t-PA and scu-PA were highly synergistic (\textit{p} < 0.01).

The synergistic effect of t-PA and scu-PA was confirmed in a preparation of coronary artery thrombosis in the dog. Whereas coronary artery reperfusion within 30 min required a minimal infusion rate of 30

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*Samama M: Personal communication."
TABLE 1
Synergism of thrombolytic agents

<table>
<thead>
<tr>
<th>End point</th>
<th>Agents studied</th>
<th>Combination</th>
<th>Dimension</th>
<th>Single agents</th>
<th>A</th>
<th>A + B</th>
<th>A</th>
<th>A + B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human plasma clot in vitro</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>25% lysis in 4 hr</td>
<td>nt-PA, rscu-PA</td>
<td></td>
<td>nM</td>
<td>0.75, 5</td>
<td>0.38, 2.5</td>
<td>1/6.5, 1</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>50% lysis in 2 hr</td>
<td>nt-PA, nsu-PA</td>
<td></td>
<td>nM</td>
<td>4.5, 23</td>
<td>3, 3</td>
<td>1/1</td>
<td>0.70, 12,20</td>
<td></td>
</tr>
<tr>
<td>50% or 100% lysis</td>
<td>nt-PA, nsu-PA</td>
<td></td>
<td>nM</td>
<td>NA, NA</td>
<td>NA, NA</td>
<td>1/4</td>
<td>1, 13,19</td>
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<tr>
<td>Rabbit jugular vein thrombosis</td>
<td></td>
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</tr>
<tr>
<td>25% lysis in 4 hr</td>
<td>nt-PA, nsu-PA</td>
<td></td>
<td>mg/kg</td>
<td>0.16, 0.70</td>
<td>0.045, 0.09</td>
<td>1/2</td>
<td>0.40, 10</td>
<td></td>
</tr>
<tr>
<td>Dog coronary artery thrombosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Reperfusion within 30 min</td>
<td>rt-PA, nsu-PA</td>
<td></td>
<td>µg/kg/min</td>
<td>30, 10</td>
<td>7.5, 2.5</td>
<td>3/1</td>
<td>0.5, 23</td>
<td></td>
</tr>
<tr>
<td>Acute myocardial infarction</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Reperfusion within 60 min</td>
<td>rt-PA, nsu-PA</td>
<td></td>
<td>mg/hr</td>
<td>40, 40</td>
<td>10, 3</td>
<td>3/1</td>
<td>0.3, 24,25</td>
<td></td>
</tr>
</tbody>
</table>

$A_s, B_s$ = equipotential dose required to obtain the end point; nt-PA = natural t-PA; rt-PA = recombinant t-PA; nsu-PA = natural scu-PA; rscu-PA = recombinant scu-PA; NA = data not available.

*p < .05; **p < .01 (level of significance as vs 1).

µg/kg/min of t-PA or of 10 µg/kg/min of scu-PA, the combination of 7.5 and 2.5 µg/kg/min, constituting an algebraic fraction of 0.5, was equipotent. In aggregate, these observations in two different species suggest that synergism of t-PA and scu-PA in vivo is not species dependent, but also that it only occurs in a relatively narrow concentration range.

Results in patients with coronary artery occlusion. A preliminary evaluation of the synergistic effect of t-PA and scu-PA has been performed in man. When t-PA and scu-PA, which individually require an intravenous dose of at least 40 mg to cause consistent coronary artery reperfusion within 1 hr, were infused in a combination of 10 and 3 mg, respectively, coronary artery reperfusion was observed in three of three patients with acute myocardial infarction. This combination represents an estimated algebraic fraction of 0.3. These initial observations on the synergistic effect of t-PA and scu-PA have been confirmed in an additional pilot study in nine patients with the use of combinations of 10 mg t-PA and 10 mg scu-PA, constituting an equipotential algebraic fraction of 0.5 (table 1).

A significant observation relating to the combined use of t-PA and scu-PA in man is the absence of systemic fibrinogen degradation. Indeed, whereas individual therapeutic doses of t-PA and scu-PA induce moderate-to-severe systemic fibrinolytic activation in a significant proportion of patients, fibrinogen breakdown has not been observed in any of the 12 patients studied to date with the combined equipotential dose of 10 mg t-PA and 3 or 10 mg scu-PA (table 1).

In conclusion, although the investigation of the synergistic effects of thrombolytic agents has only recently been initiated, encouraging preliminary results in patients justify a more elaborate clinical evaluation of the potential and limitations of synergism. The available data suggest that synergism of t-PA and scu-PA only occurs in a relatively narrow dose range that may however be sufficient to result in a two- to 5-fold reduction of the total necessary dose, and that the reduced dose combination is associated with less systemic fibrinolytic activation. To reach valid conclusions, continued investigation of synergism should be carried out with the use of adequate investigational procedures with respect to both the experimental design and data analysis.

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D Collen

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