Thrombolytic Profiles of Clot-Targeted Plasminogen Activators
Parameters Determining Potency and Initial and Maximal Rates

Paul Holvoet, PhD; Maria Dewerchin, PhD; Jean Marie Stassen; Henri Roger Lijnen, PhD; Tom Tollenaere; Patrick J. Gaffney, PhD; and Désiré Collen, MD, PhD

Background. Targeting of plasminogen activators to the thrombus by means of fibrin-specific monoclonal antibodies may enhance their thrombolytic potency. The kinetics of clot binding of two human fibrin-specific monoclonal antibodies (MA-12B3 and MA-15C5) and of clot lysis with their chemical 1:1 stoichiometric complexes with recombinant single-chain urokinase-type plasminogen activator (rscu-PA) (rscu-PA/MA-12B3 and rscu-PA/MA-15C5) were determined in hamsters and rabbits. Thrombolytic potencies, maximal rates of clot lysis, and the duration of the lag phases before clot lysis of the antibody/rscu-PA conjugates were compared with those of rscu-PA and tissue-type plasminogen activator (rt-PA).

Methods and Results. Bolus injection of 7.5 μg of 125I-labeled antibody in rabbits with an extracorporeal arteriovenous loop containing a 0.3-mL human plasma clot produced clot-to-blood ratios of 6.6±1.0 (mean±SEM) for MA-12B3 and 1.1±0.15 for MA-15C5 (p<0.001 versus MA-12B3) within 6 hours. Progressive digestion of the clot did not alter the binding of MA-12B3 but resulted in as much as a 10-fold increase of the binding of MA-15C5. The conjugates infused intravenously over 90 minutes in hamsters with a human plasma clot in the pulmonary artery produced dose-related in vivo clot lysis. Thrombolytic potencies (maximal slope of the percent lysis versus dose in milligrams of u-PA equivalent per kilogram body weight) were 2,500±440 for rscu-PA/MA-12B3, 3,600±640 for rscu-PA/MA-15C5 (p=NS vs. rscu-PA/MA-12B3), 60±8 for rscu-PA (p<0.001 versus both conjugates), and 380±66 for rt-PA (p<0.001 versus both conjugates). The plasma clearances of the conjugates were fourfold to sixfold slower than those of rscu-PA and rt-PA. Maximal rates of clot lysis, determined by continuous external radioisotope scanning over the thorax, were 0.90±0.13%, 0.91±0.17%, 0.84±0.12%, and 1.1±0.16% lysis per minute for rscu-PA/MA-12B3, rscu-PA/MA-15C5, rscu-PA, and rt-PA, respectively; these maximal rates were obtained with 0.016, 0.016, 1.0, and 0.25 mg/kg, respectively, and were associated with minimal lag phases of 18±3.2, 28±4.9, 34±3.7, and 25±3.9 minutes, respectively.

Conclusions. The thrombolytic potency of the rscu-PA/antifibrin conjugates is determined by their clearance, as well as by rate and extent of initial binding to clots and by changes in binding during clot lysis. Clot targeting of rscu-PA with fibrin-specific antibodies increases its thrombolytic potency but does not alter the maximal rate or the minimal lag phase of clot lysis. These parameters appear to be independent of the nature of the plasminogen activator and of targeting. (Circulation 1993;87:1007–1016)

Key Words • kinetics • thrombolysis • acute myocardial infarction • plasminogen activators

Fibrin-specific monoclonal antibodies may be clinically useful reagents for the diagnosis and treatment of thrombosis.1 Fibrin-directed monoclonal antibodies have been evaluated for in vivo thrombus imaging by external radioisotope scanning2–7 and for targeting of thrombolytic agents to a thrombus.8–18 A recombinant chimeric plasminogen activator (rscu-PA-32k/59D8) consisting of recombinant single-chain urokinase-type plasminogen activator (rscu-PA) fused to the murine monoclonal antibody 59D8, which is directed against an aminoterminal epitope in the β-chain of fibrin,3 was found to have a sixfold higher thrombolytic potency than rscu-PA.16 Chemical complexes of the monoclonal antibody MA-15C54 directed against a neoantigenic epitope in fragment D-dimer of human cross-linked fibrin, and rscu-PA had a sixfold enhanced potency in venous thrombosis models in rabbits14 and baboons.15 In addition, the recombinant chimeric plasminogen activator rscu-PA-32k/MA-15CSHu, which consists of humanized MA-15C5 (MA-15CSHu) and a 32-kd single-chain urokinase-type plasminogen activator comprising amino acids Leu41–Leu41 (scu-PA-32k),17 had an 11-fold higher thrombolytic potency than rscu-PA in rabbits with a jugular vein clot prepared from human plasma.18

From the Center for Thrombosis and Vascular Research (P.H., M.D.), J.M.S., H.R.L., D.C.) and the Laboratory for Neuro- and Psychophysiology (T.T.), University of Leuven, Belgium; and the National Institute for Biological Standards and Control (P.J.G.), Hertfordshire, UK.

Address for correspondence: Dr. P. Holvoet, Center for Thrombosis and Vascular Research, K.U. Leuven, Campus Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium.

Received July 23, 1992; revision accepted November 24, 1992.
Clot targeting of thrombolytic agents with antifibrin antibodies may be hampered by a low density of the specific epitopes at the clot surface or by changing binding profiles during clot maturation or lysis.\textsuperscript{19} Therefore, in the present in vivo study in hamsters and rabbits, we determined the clot-binding and the plasminogen activator–targeting properties of another fibrin–specific monoclonal antibody, MA-12B3, which is directed against an epitope localized in a 27-amino-acid region (Lys\textsuperscript{78}–Asn\textsuperscript{104}) spanning the carboxy terminal end of fragment E and the amino terminal end of fragment D in the \(\alpha\)-chain of fibrin.\textsuperscript{20} We hypothesized that this epitope would be initially exposed at the clot surface and remain associated with the core of the thrombus during plasmin-mediated fibrin digestion. To delineate the contributions of the rate and extent of initial binding to native clots and of alterations in the binding during clot lysis to the overall targeting capacity of antibody/plasminogen activator complexes, the kinetics of the binding of MA-12B3 and MA-15C5 to the surface of native and partially digested human plasma clots were determined as well as the time course of clot lysis with rsu-PA/MA-12B3 and rsu-PA/MA-15C5. To delineate the effect of clot targeting on thrombolytic potency, maximal rate of lysis, and time required to initiate lysis, the kinetics of clot lysis with rsu-PA, rsu-PA/antifibrin conjugates, and rt-PA were compared.

**Methods**

**Reagents**

rsu-PA prepared by expression of cDNA encoding scu-PA in *E. coli* was a kind gift from Grünenthal AG (Aachen, Germany). Urokinase for in vivo plasma clot digestion was from SeroNo (Freiburg, Germany). The rt-PA used was Activase\textsuperscript{8}, kindly supplied by Genentech Inc. (South San Francisco, Calif.). MA-12B3 and MA-15C5 were obtained and characterized as described elsewhere.\textsuperscript{4,20} MA-12B3 or MA-15C5 was conjugated with the heterobifunctional cross-linking reagent N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP)\textsuperscript{21} as described previously.\textsuperscript{13} The resulting rsu-PA/MA-12B3 and rsu-PA/MA-15C5 conjugates were purified by affinity chromatography on immobilized MA-4D1E8, a monoclonal antibody specific for urokinase,\textsuperscript{22} followed by gel filtration.

Human fibrinogen labeled with \(^{125}\text{I}\) was purchased from Amersham Research Products (Amersham, Brussels, Belgium). The monoclonal antibodies MA-12B3 and MA-15C5 were labeled by the Bolton and Hunter method.\textsuperscript{23}

**Analytical Techniques**

u-PA–related antigen and t-PA antigen were determined using specific ELISAs.\textsuperscript{22,24} Determinations of \(\alpha_2\)-antiplasmin and fibrinogen in plasma were performed with the Automated Coagulation Laboratory (ACL, Instrumentation Laboratory, Milan, Italy) as recommended by the manufacturer, with minor modifications. The affinity constants for immobilized fibrin of MA-12B3, MA-15C5, rsu-PA/MA-12B3, and rsu-PA/MA-15C5 were calculated as described earlier.\textsuperscript{25} Specific fibrinolytic activities were measured on fibrin plates\textsuperscript{26} by comparison with the International Reference Preparation for Urokinase.

**Binding of \(^{125}\text{I}\)-Labeled MA-12B3 and MA-15C5 to Intact and Partially Digested Human Plasma Clots in a Rabbit Extracorporeal Circulation Model**

Human plasma clots (0.3 mL) were produced in a 1-mL syringe. \(^{125}\text{I}\)-Labeled human fibrinogen (Amersham) containing \(~50,000\,\text{cpm}\) (approximately 0.05 \(\mu\text{Ci}\)) was mixed with pooled human plasma to which 10 NIH units/mL of thrombin (Sigma, St. Louis, Mo.) and 34 mM CaCl\(_2\) were added. The mixture was then aspirated in the syringe, and the clot was aged for 60 minutes. The syringe was inserted into an extracorporeal loop connecting the femoral artery with the femoral vein of a rabbit. Further incorporation of fibrinogen into the clot was prevented with heparin (Liquemin, F. Hoffmann-La Roche, Basel, Switzerland) given as a bolus of 300 IU/kg, followed by an intravenous infusion of 60 IU · kg\(^{-1}\) · hr\(^{-1}\) over 6 hours. Adhesion of platelets to the clot was prevented by injection of 7.5 mg/kg ridogrel, a combined thromboxane synthase inhibitor/endoperoxide receptor antagonist\textsuperscript{27} (Janssen Research Foundation, Beerse, Belgium), 30 minutes before the start of the experiment. \(^{125}\text{I}\)-Labeled MA-12B3 or MA-15C5 (7.5 \(\mu\text{g}\) per rabbit representing 25 \(\mu\text{Ci}\) \(^{125}\text{I}\)) was injected via a marginal ear vein. In the first series of experiments, the binding of antibody to intact human plasma clots was determined by measuring the radioisotope bound to the clot, which was recovered 6 hours after the injection of labeled antibody. In all subsequent experiments, the time course of the binding of \(^{125}\text{I}\)-labeled antibody to the clot in the syringe was monitored with a scintillation crystal (Model 3 M.S-3, Bicron, Newbury, Ohio) connected to a multichannel photomultiplier (Model S-100, Canberra, Meridan, Conn.). The \(^{125}\text{I}\) incorporated in the plasma clot as \(^{125}\text{I}\)-labeled fibrinogen to allow determination of the extent of digestion represented approximately 0.2% of the amount of the injected \(^{125}\text{I}\)-labeled antibody and <10% of the \(^{125}\text{I}\)-labeled antibody bound to the clot. Rabbit plasma clots that do not cross-react with the human fibrin–specific antibodies were prepared in the same way as the human plasma clots and were incorporated in a second syringe in the same extracorporeal loop. The radioactivity that bound to the rabbit plasma clots was subtracted as aspecific binding from the radioactivity that bound to the human plasma clots. The number of clot-bound antibody molecules was calculated from the amount of radioactivity that specifically bound to the human plasma clot from the specific radioactivities of the \(^{125}\text{I}\)-labeled antibody preparations. Partial digestion of the human plasma clots in the extracorporeal loop was produced by intravenous infusion of urokinase (0.15, 0.3, or 0.6 mg/kg for 30 minutes, respectively), resulting in a residual clot volume of 81±0.6%, 54±2%, and 35±0.7% of the original clot volume, respectively, as determined by external scanning of residual \(^{125}\text{I}\)-fibrin in the extracorporeal loop. The stability of the partially digested clots after the end of the urokinase infusion was confirmed by monitoring of their radioisotope content for 30 minutes before injection of the \(^{125}\text{I}\)-labeled antibody. The individual clot-binding data, recorded at 5-minute intervals, were fitted with an exponentially transformed sigmoidal function:

\[
y = \frac{c}{1 + e^{a(x-b)}}
\]
according to Marquart,28 using the statistical program GRAFIT (Erithacus Ltd., Middlesex, UK). The mean±SEM values of the following parameters were calculated: \( r \), maximal rate of binding (expressed in \( 10^{10} \text{ molecules/min} \)), \( t_l \), lag phase (expressed in minutes), defined as the intercept of the linear part of the curve with the abscissa and calculated as \( (a−2)/b \); and \( t_2 \), the time (minutes) at which the binding is maximal and calculated as \( (a+2)/b \).

**Pharmacokinetics of \(^{125}\text{I}-\text{Labeled MA-12B3 and MA-15C5**}

The pharmacokinetic properties of MA-15C5 and MA-12B3 in rabbits were determined by measurement of the residual plasma radioactivity after bolus injection of 7.5 \( \mu \text{g} \) of \(^{125}\text{I}-\text{labeled antibody. Blood samples were taken at times} 1, 2, 5, 10, 20, 30, 60, 120, 180, 240, 300, \) and 360 minutes for measurement of residual radioactivity. The results were plotted on semilogarithmic paper and fitted with a sum of the two exponential terms \( C(t) = A e^{-\alpha t} + B e^{-\beta t} \) by graphical curve peeling.29 Therefore, the linear portion of the curve was extrapolated to yield the intercept \( B \). This line had a slope \( -\beta \). The extrapolated values were subtracted from the initial values, and the corrected values were fitted with a line that had a slope \( -\alpha \) and an ordinate intercept \( A \). The following clearance parameters were calculated from the coefficients \( (A \) and \( B \)) and exponents \( (\alpha \) and \( \beta \)) describing the disposition of MA-15C5 or MA-12B3 from plasma, using standard formulas derived by Gibaldi and Perrier29: volume of the central compartment = dose/(\( A + B \)); total volume of distribution = dose/B; extrapolated area under the curve \( \text{AUC} = A/\alpha + B/\beta \); plasma clearance = dose/AUC; \( t_1/2 \alpha = (\ln 2)/\alpha \); and \( t_1/2 \beta = (\ln 2)/\beta \).

**Thrombolysis of \(^{125}\text{I}-\text{Fibrin–Labeled Human Plasma Clots in a Hamster Pulmonary Embolism Model**}

The thrombolytic properties of rscu-PA/MA-12B3, rscu-PA/MA-15C5, rscu-PA, and rt-PA were determined in hamsters with an experimental pulmonary embolus consisting of a human plasma clot injected via the jugular vein, as described previously.32 Thrombolytic agents were infused intravenously over 60 minutes, and lysis was measured 30 minutes after the end of the infusion as the difference between the radioactivity initially incorporated in the clot and the residual radioactivity in the lungs and the heart. Alternatively, the release of the radioactivity from the clot was measured continuously as was described earlier for the rabbit jugular vein thrombosis model.33 Blood samples were collected before the start of the infusion at 60 minutes (for determination of u-PA–related antigen) and at 90 minutes (for determination of residual fibrinogen and \( \alpha_2 \)-antiplasmin levels).

**Analysis of the Thrombolysis Data**

At the end of the thrombolysis experiments in rabbits, the clot was removed from the jugular vein, and the extent of thrombolysis was calculated from the disappearance of radioactivity from the clot. At the end of the thrombolysis experiments in hamsters, the lungs were removed, and the residual radioactivity in the lungs of the hamsters was measured. The extent of thrombolysis was derived from the disappearance of radioactivity from the lungs. The relative thrombolytic potency and specific thrombolytic activity of the compounds in hamsters and rabbits were determined from the dose–response curves derived by fitting of the individual dose–response data (percent lysis versus dose in mg/kg or percent lysis versus steady-state u-PA–related antigen in \( \mu \text{g/mL} \)) with an exponentially transformed sigmoidal function:

\[
y = \frac{100c}{1 + e^{-(a-b)\cdot y}}
\]

using the statistical program GRAFIT (Erithacus Ltd., Middlesex, UK). Mean±SEM values of the parameters \( c \) (maximal lysis in percent), \( b \) (dose in mg/kg or in \( \mu \text{g/mL} \) plasma antigen at which the rate of lysis is maximal), and \( z = (ac/4) \cdot e^{b} \) (maximal rate of lysis, expressed as percent lysis per mg/kg compound infused or as percent lysis per \( \mu \text{g/mL} \) plasma antigen) were determined.34 The significance of the differences between these parameters was determined using Student’s \( t \) test.

To determine the time course of thrombolysis in hamsters, the disappearance of radioactivity from the lungs was monitored continuously by external radioisotope scanning over the thorax.23,32 The rates of thrombolysis obtained with the different compounds in hamsters were determined by fitting the individual data (percent lysis versus time in minutes) with an exponentially transformed sigmoidal function:

\[
y = \frac{c}{1 + e^{-(a-b)\cdot y}}
\]

using the statistical program GRAFIT as described above for determining the clot-binding parameters. Mean±SEM values of the following parameters were
TABLE 1. Binding of 125I-Labeled MA-12B3 and MA-15C5 to Intact or Partially Degraded Human Plasma Clots in Rabbits

<table>
<thead>
<tr>
<th>Residual clot size (%)</th>
<th>No. of bound molecules ($\times 10^{10}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA-12B3</td>
<td>MA-15C5</td>
</tr>
<tr>
<td>100 (16)</td>
<td>19$\pm$5.0 (8)</td>
</tr>
<tr>
<td>81$\pm$0.6 (8)</td>
<td>13$\pm$5.0 (4)</td>
</tr>
<tr>
<td>54$\pm$2.0 (10)</td>
<td>12$\pm$2.5 (5)</td>
</tr>
<tr>
<td>35$\pm$0.7 (10)</td>
<td>12$\pm$2.0 (3)</td>
</tr>
</tbody>
</table>

Data represent mean$\pm$SEM of the number of experiments indicated between brackets.

calculated: $r$, maximal rate of lysis (expressed in percent per minute), as the slope at the inflection point of the curve; lag-phase $t_1$ (expressed in minutes), defined as the intercept of the linear part of the curve with the abscissa and calculated as $(a-2)/b$; and $t_2$, the time (minutes) at which maximal lysis is obtained and calculated as $(a+2)/b$.

Results

Binding of 125I-Labeled MA-12B3 and MA-15C5 to Intact and Partially Degraded Human Plasma Clots Inserted in an Extracorporeal Arteriovenous Loop in Rabbits

Within 6 hours after the injection of 7.5 µg of 125I-labeled antibody, 19$\pm$5.0$\times 10^{10}$ (mean$\pm$SEM, $n=8$) molecules of MA-12B3 bound to the human plasma clot, resulting in a clot-to-blood ratio of 6.6$\pm$1.0. A sixfold-lower number of 125I-labeled MA-15C5 molecules (2.9$\pm$0.8$\times 10^{10}$, $n=8$, $p=0.007$ versus MA-12B3) bound to the clot, resulting in a clot-to-blood ratio of 1.1$\pm$0.15 ($p<0.001$ versus MA-12B3) (Table 1). Intravenous infusion of 0.15, 0.30, or 0.60 mg/kg urokinase over 30 minutes produced digestion of the clots to 81$\pm$0.6%, 54$\pm$2%, and 35$\pm$0.7% of baseline, respectively. Predigestion resulted in binding of 13$\pm$5.0$\times 10^{10}$, 12$\pm$2.5$\times 10^{10}$, and 12$\pm$2.0$\times 10^{10}$ molecules, respectively, for MA-12B3 and 8.5$\pm$1.7$\times 10^{10}$, 13$\pm$0.8$\times 10^{10}$, and 25$\pm$3.6$\times 10^{10}$ molecules, respectively, for MA-15C5 after correction for clot volume reduction.

Figure 1 illustrates the course of the binding of 125I-labeled MA-12B3 and MA-15C5 to intact human plasma clots inserted into the arteriovenous loop in rabbits. The clot-binding data were fitted with an exponentially transformed sigmoidal function as described in "Methods." The values for parameters $r$, $t_1$, and $t_2$ for binding of labeled MA-12B3 and MA-15C5 are illustrated in Table 2. The maximal rates of binding ($r$) were 2.2$\pm$0.5$\times 10^{10}$ molecules per minute (mean$\pm$SEM; $n=5$) for MA-12B3 and 60$\pm$28$\times 10^{10}$ molecules per minute ($n=5$) for MA-15C5. Maximal rates of binding were reached within 5 minutes after injection with both MA-12B3 and MA-15C5. The maximal extent of binding was reached at 350$\pm$17 minutes with MA-12B3 and at 10$\pm$2.0 minutes with MA-15C5 ($p<0.001$ versus MA-12B3).

Pharmacokinetics of MA-12B3 and MA-15C5 in Rabbits

125I-Labeled MA-12B3 and MA-15C5 were cleared from the blood with initial half-lifes ($t_1/2a$) of 90 minutes, with terminal half-lifes ($t_1/2b$) of 30 hours and with clearances of 0.093$\pm$0.004 mL·min$^{-1}$.

Figure 1. Time course of the binding of 125I-labeled MA-12B3 (●) and MA-15C5 (▲) to intact human plasma clots. The data, expressed as number of bound molecules ($\times 10^{10}$), are mean$\pm$SEM of five independent experiments.

Fibrin Affinity and Specific Fibrinolytic Activity of the rscu-PA/MA-12B3 and rscu-PA/MA-15C5 Conjugates

The affinity constants ($K_a$) of rscu-PA/MA-12B3 and MA-12B3 for immobilized fibrin were 0.9$\pm$0.2$\times 10^{9}$ and 1.6$\pm$0.7$\times 10^{9}$ M$^{-1}$, respectively (mean$\pm$SEM, $n=6$). The rscu-PA/MA-15C5 conjugate bound to immobilized fragment D-dimer with $K_a$ was 5$\times 10^{9}$ M$^{-1}$ (mean$\pm$SEM, $n=4$) compared with 1$\times 10^{10}$ M$^{-1}$ for native MA-15C5.

The specific fibrinolytic activities as measured on fibrin plates were 97,000$\pm$42,000 IU/mg u-PA (mean$\pm$SEM, $n=4$) for rscu-PA/MA-12B3 and 100,000$\pm$17,000 IU/mg u-PA ($n=4$) for rscu-PA/MA-15C5 compared with 120,000$\pm$20,000 IU/mg u-PA ($n=4$) for rscu-PA.

Lysis of 125I-Labeled Human Plasma Clots in the Rabbit Jugular Vein Thrombosis Model

Dose–response data of clot lysis with rscu-PA/MA-12B3, rscu-PA/MA-15C5, and rscu-PA in rabbits with a 125I-fibrin–labeled human plasma clot produced in the jugular vein are summarized in Table 3. Clot lysis measured 30 minutes after the end of the infusion of saline into six control animals was 8$\pm$1% (mean$\pm$SEM; $n=6$). Fibrinogen and $\alpha_2$-antiplasmin levels at the end of the experiment were 110$\pm$10% and 96$\pm$4% of the baseline values, respectively. With rscu-PA/MA-12B3 at

TABLE 2. Time Course of Binding of 125I-Labeled MA-12B3 and MA-15C5 to Native Human Plasma Clots in the Rabbit Extracorporeal Loop

<table>
<thead>
<tr>
<th></th>
<th>Maximal rate of binding; $r$ (10$^{10}$ molecules/min)</th>
<th>Time of maximal extent of binding; $t_2$ (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA-12B3</td>
<td>2.2$\pm$0.5</td>
<td>350$\pm$17</td>
</tr>
<tr>
<td>MA-15C5</td>
<td>60$\pm$28</td>
<td>10$\pm$2.0</td>
</tr>
</tbody>
</table>

Data represent mean$\pm$SEM for five experiments each and were obtained by fitting of the binding data with an exponentially transformed sigmoidal function $y=\frac{c}{1+e^{-(x-a)/b}}$ (see "Methods").
TABLE 3. Clot Lysis and Hemostasis Parameters Obtained With rscu-PA/MA-12B3, rscu-PA/MA-15C5, or rscu-PA in Rabbits With a Jugular Vein Thrombosis Consisting of Human Plasma

<table>
<thead>
<tr>
<th>Dose (mg u-PA/kg)</th>
<th>n</th>
<th>Clot lysis at 270 minutes (%)</th>
<th>Residual fibrinogen (% of baseline)</th>
<th>Residual α2-antiplasmin (% of baseline)</th>
<th>Steady-state plasma antigen level* (ng/mL)</th>
<th>Clp† (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>6</td>
<td>8±1</td>
<td>110±10</td>
<td>96±4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>rscu-PA/MA-12B3</td>
<td>0.032</td>
<td>3</td>
<td>28±8</td>
<td>110±2</td>
<td>93±8</td>
<td>91±16</td>
</tr>
<tr>
<td></td>
<td>0.063</td>
<td>3</td>
<td>41±2</td>
<td>100±4</td>
<td>97±1</td>
<td>140±25</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>3</td>
<td>53±7</td>
<td>110±3</td>
<td>90±6</td>
<td>370±27</td>
</tr>
<tr>
<td></td>
<td>0.250</td>
<td>2</td>
<td>74±10</td>
<td>98±2</td>
<td>82±4</td>
<td>840±130</td>
</tr>
<tr>
<td>rscu-PA/MA-15C5</td>
<td>0.063</td>
<td>4</td>
<td>16±3</td>
<td>100±3</td>
<td>91±4</td>
<td>240±37</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>4</td>
<td>47±6</td>
<td>100±2</td>
<td>95±2</td>
<td>530±65</td>
</tr>
<tr>
<td></td>
<td>0.250</td>
<td>4</td>
<td>57±5</td>
<td>100±3</td>
<td>91±3</td>
<td>1,000±130</td>
</tr>
<tr>
<td></td>
<td>0.500</td>
<td>4</td>
<td>73±5</td>
<td>96±6</td>
<td>77±3</td>
<td>2,000±94</td>
</tr>
<tr>
<td>rscu-PA</td>
<td>1.0</td>
<td>5</td>
<td>15±2</td>
<td>100±5</td>
<td>87±5</td>
<td>500±92</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>5</td>
<td>38±5</td>
<td>93±5</td>
<td>80±3</td>
<td>830±140</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>6</td>
<td>73±6</td>
<td>87±6</td>
<td>69±4</td>
<td>2,200±350</td>
</tr>
</tbody>
</table>

Data represent mean±SEM.
*Steady-state plasma antigen levels are u-PA-related antigen levels in plasma samples taken at the end of the infusion.
†The plasma clearance (Clp) was calculated as the ratio of the infusion rate (µg/min) and the steady-state plasma concentration of antigen (µg/mL).

Doses of 0.032, 0.063, 0.125, and 0.25 mg/kg u-PA, lysis was 28±8% (n=3), 41±2% (n=3), 53±7% (n=3), and 74±10% (n=2), respectively. The corresponding fibrinogen levels were 110±2%, 100±4%, 110±3%, and 98±2%, respectively. The α2-antiplasmin levels were 93±8%, 97±1%, 90±6%, and 82±4% of the baseline value, respectively. With rscu-PA/MA-15C5 at doses of 0.063, 0.125, 0.25, and 0.50 mg/kg u-PA, lysis was 16±3% (n=4), 47±6% (n=4), 57±5% (n=4), and 73±5% (n=4), respectively. The corresponding levels of fibrinogen were 100±3%, 100±3%, 100±3%, and 99±6%, and the α2-antiplasmin levels were 91±4%, 95±2%, 91±3%, and 77±3% of the baseline value, respectively. With rscu-PA at doses of 1.0, 2.0, and 4.0 mg/kg, lysis was 15±2% (n=5), 38±5% (n=6), and 73±6% (n=6), respectively. The corresponding levels of fibrinogen were 100±5%, 93±5%, and 87±6%, and α2-antiplasmin levels were 87±5%, 80±3%, and 69±4% of the baseline values, respectively.

Parameters determining the thrombolytic potency of the study compounds were derived by fitting the dose-response data, expressed as percent lysis per milligram u-PA equivalent administered per kilogram body weight, with the exponentially transformed sigmoidal function. Values of z, b, and c are summarized in Table 4. With rscu-PA/MA-12B3, the maximal rate of lysis was 1.8-fold higher (p=NS) than for rscu-PA/MA-15C5 (z values of 480±100% and 270±52% per milligram u-PA/kg, respectively) and 11-fold higher (p<0.001) than for rscu-PA (z=43±8% per milligram u-PA/kg). The maximal rate of lysis was obtained at a dose of 0.056±0.013 mg u-PA/kg of rscu-PA/MA-12B3, at 0.13±0.02 mg u-PA/kg of rscu-PA/MA-15C5 (p<0.01 versus rscu-PA/MA-12B3), and at 2.0±0.01 mg u-PA/kg of rscu-PA (p<0.001 versus both conjugates).

Specific thrombolytic activity parameters were derived by fitting the dose–response data, expressed as percent lysis per microgram per milliliter steady-state plasma u-PA–related antigen with the exponentially transformed sigmoidal function, yielding the corresponding c', z', and b' values.

TABLE 4. Comparative Thrombolytic Potency and Specific Thrombolytic Activity of rscu-PA/MA-12B3, rscu-PA/MA-15C5, and rscu-PA in the Rabbit Jugular Vein Human Plasma Clot Model

<table>
<thead>
<tr>
<th>Thrombolytic potency</th>
<th>Specific thrombolytic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>z</td>
</tr>
<tr>
<td>rscu-PA/MA-12B3</td>
<td>17</td>
</tr>
<tr>
<td>rscu-PA/MA-15C5</td>
<td>22</td>
</tr>
<tr>
<td>rscu-PA</td>
<td>22</td>
</tr>
</tbody>
</table>

Data represent mean±SEM obtained by fitting the individual dose response data of lysis versus dose (z, b, c) or lysis versus steady-state plasma antigen level (z', b', c') with an exponentially transformed sigmoidal function \( y = \frac{100c}{1 + e^{-a(z-z')}} \) with z = \( \frac{b c'}{b' c} \).

z,z': Maximal rate of lysis expressed as percent lysis per mg/kg dose (z), or percent lysis per µg/mL plasma antigen level (z').
b,b': Dose expressed in mg/kg (b), or plasma antigen level expressed in µg/mL plasma (b'), at which the rate of clot lysis is maximal.
c,c': Maximal extent of lysis, expressed in percent.
Table 5. Clot Lysis and Hemostasis Parameters Obtained With rscu-PA/MA-12B3, rscu-PA/MA-15C5, rscu-PA, or rt-PA in Hamsters With a Pulmonary Embolus Consisting of a Human Plasma Clot

<table>
<thead>
<tr>
<th>Dose (mg u-PA/kg)</th>
<th>n</th>
<th>Clot lysis at 90 minutes (%)</th>
<th>Residual fibrinogen (% of baseline)</th>
<th>Residual α₁-antiplasmin (% of baseline)</th>
<th>Steady-state plasma antigen level* (ng/mL)</th>
<th>CLp† (mL·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>19±2</td>
<td>140±11</td>
<td>110±12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rscu-PA/MA-12B3</td>
<td>0.004</td>
<td>6</td>
<td>29±2</td>
<td>95±5</td>
<td>100±7</td>
<td>19±1</td>
</tr>
<tr>
<td></td>
<td>0.008</td>
<td>6</td>
<td>44±4</td>
<td>110±8</td>
<td>120±8</td>
<td>39±5</td>
</tr>
<tr>
<td></td>
<td>0.016</td>
<td>6</td>
<td>53±5</td>
<td>120±9</td>
<td>100±10</td>
<td>72±11</td>
</tr>
<tr>
<td></td>
<td>0.032</td>
<td>6</td>
<td>73±6</td>
<td>130±6</td>
<td>110±20</td>
<td>130±7</td>
</tr>
<tr>
<td>rscu-PA/MA-15C5</td>
<td>0.008</td>
<td>6</td>
<td>33±2</td>
<td>150±11</td>
<td>100±8</td>
<td>22±8</td>
</tr>
<tr>
<td></td>
<td>0.016</td>
<td>8</td>
<td>72±6</td>
<td>130±6</td>
<td>120±11</td>
<td>48±8</td>
</tr>
<tr>
<td></td>
<td>0.032</td>
<td>7</td>
<td>76±8</td>
<td>140±13</td>
<td>110±12</td>
<td>150±25</td>
</tr>
<tr>
<td></td>
<td>0.063</td>
<td>3</td>
<td>85±4</td>
<td>110±13</td>
<td>140±16</td>
<td>230±13</td>
</tr>
<tr>
<td>rscu-PA</td>
<td>0.125</td>
<td>3</td>
<td>25±2</td>
<td>130±6</td>
<td>130±37</td>
<td>97±3</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>7</td>
<td>30±3</td>
<td>150±16</td>
<td>120±12</td>
<td>220±24</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>5</td>
<td>31±7</td>
<td>170±15</td>
<td>100±5</td>
<td>370±26</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>8</td>
<td>73±6</td>
<td>140±10</td>
<td>110±5</td>
<td>980±170</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>5</td>
<td>82±7</td>
<td>130±5</td>
<td>66±3</td>
<td>2,100±300</td>
</tr>
<tr>
<td>rt-PA</td>
<td>0.032</td>
<td>4</td>
<td>32±7</td>
<td>140±10</td>
<td>100±9</td>
<td>21±3</td>
</tr>
<tr>
<td></td>
<td>0.063</td>
<td>4</td>
<td>44±5</td>
<td>120±10</td>
<td>95±5</td>
<td>35±6</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>4</td>
<td>66±5</td>
<td>120±15</td>
<td>107±7</td>
<td>86±13</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>4</td>
<td>83±4</td>
<td>130±5</td>
<td>100±8</td>
<td>190±42</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>4</td>
<td>84±6</td>
<td>120±19</td>
<td>100±8</td>
<td>320±61</td>
</tr>
</tbody>
</table>

Data represent mean±SEM.

*Steady-state plasma antigen levels are u-PA–related antigen levels in plasma samples taken at the end of the infusion.

†The plasma clearance (CLp) was calculated as the ratio of the infusion rate (μg/min) and the steady-state plasma concentration of antigen (μg/mL).

190±62% and 86±20% lysis per μg/mL steady-state plasma u-PA–related antigen, respectively) and was 3.1-fold higher (p=0.025) than for rscu-PA (61±9% lysis per μg/mL u-PA antigen). The maximal rate of lysis was obtained at a steady-state plasma u-PA antigen concentration of 0.12±0.03 μg/mL of rscu-PA/MA-12B3 compared with 0.39±0.07 μg/mL of rscu-PA/MA-15C5 (p=0.003) and 1.1±0.08 μg/mL of rscu-PA (p<0.001).

Pharmacokinetics of rscu-PA/MA-12B3 and rscu-PA/MA-15C5 in Rabbits

Plasma clearances derived from the steady-state plasma u-PA antigen levels at the end of the infusion were 3.8 mL·min⁻¹ for rscu-PA/MA-12B3, 2.6 mL·min⁻¹ for rscu-PA/MA-15C5, and 22 mL·min⁻¹ for rscu-PA (Table 3).

Lysis of 125I-Fibrin–Labeled Human Plasma Clots in the Hamster Pulmonary Embolism Model

The thrombolytic potencies of rscu-PA/MA-12B3, rscu-PA/MA-15C5, rscu-PA, and rt-PA were determined in a hamster pulmonary embolism model in which the pulmonary clot was prepared from normal human plasma. Dose–response data are summarized in Table 5. Lysis measured 30 minutes after the end of the infusion of saline into 12 control animals was 19±2% (mean±SEM; n=12). Fibrinogen levels and α₁-antiplasmin levels at the end of the experiment were not different from the baseline values. With rscu-PA/MA-12B3 at doses of 0.004, 0.008, 0.016, and 0.032 mg/kg, lysis was 29±2% (n=6), 44±4% (n=6), 53±5% (n=6), and 73±6% (n=6), respectively. The corresponding fibrinogen levels and α₁-antiplasmin levels were not different from baseline values. With rscu-PA/MA-15C5 at doses of 0.008, 0.016, 0.032, and 0.063 mg/kg, lysis was 33±2% (n=6), 72±6% (n=8), 76±8% (n=7), and 85±4% (n=3), respectively. Fibrinogen levels and α₁-antiplasmin levels at the end of the experiment again were not different from baseline values. With rt-PA at doses of 0.125, 0.25, 0.50, 1.0, and 2.0 mg/kg, lysis 30 minutes after the end of the infusion was 25±2% (n=3), 30±3% (n=7), 31±7% (n=5), 73±6% (n=8), and 82±7% (n=5), respectively. Fibrinogen levels and α₁-antiplasmin levels at the end of the experiment again were not different from baseline values. With rt-PA at doses of 0.032, 0.063, 0.125, 0.25, and 0.50 mg/kg, lysis was 32±7% (n=4), 44±5% (n=4), 66±5% (n=4), 83±4% (n=4), and 84±6% (n=4), respectively. Fibrinogen levels and α₁-antiplasmin levels at the end of the experiment again were not different from baseline values.

The dose–response data expressed as percent lysis per mg/kg u-PA equivalent administered were fitted with the exponentially transformed sigmoidal function as described previously. Values of c, z, and b are...
summarized in Table 6. With rscu-PA/MA-12B3, the maximal rate of lysis was 1.4-fold lower (p=NS) than for rscu-PA/MA-15C5 (z values of 2,500±440% and 3,600±640% lysis per mg u-PA/kg, respectively), 42-fold higher (p<0.001) than for rscu-PA (z=60±8% lysis per mg u-PA/kg), and 6.6-fold higher (p<0.001) than for rt-PA (z=380±66% lysis per mg t-PA). The maximal rate of lysis was obtained at similar doses of rscu-PA/MA-12B3 and of rscu-PA/MA-15C5 (0.007 mg u-PA/kg of both rscu-PA/MA-12B3 and rscu-PA/MA-15C5) compared with 0.51±0.09 mg u-PA/kg of rscu-PA (p<0.001) and to 0.058±0.006 mg t-PA/kg of rt-PA (p<0.001). The dose–response data for rscu-PA/MA-12B3, rscu-PA/MA-15C5, and rscu-PA are illustrated in Figure 2.

The dose–response data expressed as percent lysis per μg/mL steady-state plasma u-PA-related antigen were also fitted with the exponentially transformed sigmoidal function as described previously, yielding the corresponding c', z', and b' values for specific thrombolytic activity (Table 6). With rscu-PA/MA-12B3 the maximal rate of lysis was threefold lower (p=0.044) than with rscu-PA/MA-15C5 (z' values of 660±150% and 1,900±610% lysis per μg/mL steady-state plasma u-PA-related antigen, respectively), was 11-fold higher (p<0.001) than for rscu-PA (65±7% lysis per μg/mL u-PA-antigen), and was similar to that for rt-PA (700±150% lysis per μg/mL t-PA antigen). The dose–response data for rscu-PA/MA-12B3, rscu-PA/MA-15C5, and rscu-PA are illustrated in Figure 2. The maximal rate of lysis was obtained at a steady-state plasma u-PA antigen concentration of 0.021±0.007 μg/mL of rscu-PA/MA-12B3 compared with 0.011±0.004 μg/mL of rscu-PA/MA-15C5 (p=NS), with 0.46±0.05 μg/mL of rscu-PA (p<0.001), and with 0.031±0.006 μg/mL of rt-PA (p=NS).

Average plasma clearances derived from the steady-state plasma u-PA antigen levels at the end of the infusion were 0.37±0.02 mL·min⁻¹ for rscu-PA/MA-12B3, 0.49±0.04 mL·min⁻¹ for rscu-PA/MA-15C5, 1.9±0.12 mL·min⁻¹ for rscu-PA, and 2.4±0.42 mL·min⁻¹ for rt-PA (Table 5).

**Time Course of 125I-Fibrin–Labeled Human Plasma Clot Lysis in the Hamster Pulmonary Embolism Model**

All compounds induced a time-dependent clot lysis. The time course of clot lysis with doses of rscu-PA (1.0 mg/kg), rscu-PA/MA-12B3 (0.016 mg/kg), and rscu-
FIGURE 3. Time course of thrombolysis in hamsters with an experimental pulmonary embolus consisting of a human plasma clot. ○, rscu-PA/MA-12B3 (at a dose of 0.016 mg/kg); ▲, rscu-PA/MA-15C5 (at a dose of 0.016 mg/kg); ■, rscu-PA (at a dose of 1.0 mg/kg) were administered by continuous infusion over 60 minutes. The extent of thrombolysis, as evidenced by disappearance of radioactivity from the thorax region, was monitored continuously by external gamma counting.

PA/MA-15C5 (0.016 mg/kg) which gave maximal lysis within 90 minutes, is illustrated in Figure 3. The curves were fitted with the exponentially transformed sigmoidal function as described in “Methods.” Maximal rates of clot lysis were 0.90±0.13% (n=4) lysis per minute for rscu-PA/MA-12B3 (p=NS versus rscu-PA), 0.91±0.17% (n=4) lysis per minute for rscu-PA/MA-15C5 (p=NS versus rscu-PA and rscu-PA/MA-12B3), and 0.84±0.12% (n=4) lysis per minute for rscu-PA, respectively. Clot lysis was associated with a lag phase of 18±3.2 minutes for rscu-PA/MA-12B3 (p=0.03 versus rscu-PA), 28±4.9 minutes for rscu-PA/MA-15C5 (p=NS versus rscu-PA and rscu-PA/MA-12B3), and 34±3.7 minutes for rscu-PA. The maximal lysis was obtained at 76±7.4, 88±2.8, and 81±5.7 minutes, respectively. Maximal rates of clot lysis were 1.1±0.48% and 1.1±0.32% lysis per minute for fourfold higher doses of rscu-PA/MA-12B3 and rscu-PA/MA-15C5, respectively. Clot lysis at higher doses of these conjugates was associated with lag phases of 22±1.1 and 29±2.4 minutes, respectively.

Because the maximal rates of clot lysis, the minimal lag phases, and the times required to obtain maximal lysis with the rscu-PA/antifibrin conjugates were not statistically different from those with rscu-PA, the different kinetic parameters for clot lysis with rt-PA were measured. The time curve could be fitted with the same exponentially transformed function (not illustrated). Maximal rate of clot lysis was 1.1±0.16, minimal lag phase was 25±3.9 minutes, and the time required to obtain maximal lysis was 77±6.0 minutes. None of these parameters was significantly different from those obtained with rscu-PA or the rscu-PA/antifibrin conjugates.

**Discussion**

In the present study, the clot-binding and plasminogen activator—targeting properties of two fibrin-specific monoclonal antibodies, MA-12B3 and MA-15C5, were compared. MA-12B3 is directed against an epitope localized in a 27-amino-acid peptide (Lys32–Asn100) of the α-chain of human fibrin, whereas MA-15C5 is directed against an epitope in fragment D-dimer of cross-linked human fibrin. Both antibodies have a similar affinity for their respective epithopes of ~10^-10 M^-1 and a similar plasma clearance.

The binding of 125I-labeled antibodies to intact human plasma clots inserted in an extracorporeal loop in rabbits was determined at 6 hours after injection of 7.5 µg of the 125I-labeled antibodies. A significantly higher number of MA-12B3 molecules compared with MA-15C5 molecules bound to intact human plasma clots (19×10^-30 and 2.9×10^-30 molecules, respectively), resulting in a significantly higher clot-to-blood ratio for MA-12B3 than for MA-15C5 (6.6 and 1.1, respectively). These data suggest that the number of exposed α-chain epitopes reacting with MA-12B3 is significantly higher in intact human plasma clots than the number of exposed fragment D-dimer epitopes reacting with MA-15C5 and that MA-12B3 might be a preferred candidate for radioimmunoscintigraphy of thrombs.

The time course of binding of MA-12B3 and MA-15C5 to human plasma clots was, however, very different. Although binding of both antibodies occurred without a lag phase, the maximal rate of binding of MA-15C5 was 28-fold higher than that of MA-12B3, whereas maximal binding was reached within 10 minutes after injection of MA-15C5 but only after 350 minutes with MA-12B3. Furthermore, progressive predigestion of the human plasma clots resulted in a

**Table 7. Time Course of Clot Lysis With rscu-PA/MA-12B3 (0.016 mg/kg), rscu-PA/MA-15C5 (0.016 mg/kg), rscu-PA (1.0 mg/kg), or rt-PA (0.25 mg/kg) in Hamsters With a Pulmonary Embolus Consisting of a Human Plasma Clot**

<table>
<thead>
<tr>
<th></th>
<th>Maximal rate of lysis: r (%) lysis/min</th>
<th>Time of lag phase: ( t_l ) (minutes)</th>
<th>Time of maximal lysis: ( t_m ) (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rscu-PA/MA-12B3</td>
<td>0.90±0.13</td>
<td>18±3.2</td>
<td>76±7.4</td>
</tr>
<tr>
<td>rscu-PA/MA-15C5</td>
<td>0.91±0.17</td>
<td>28±4.9</td>
<td>88±2.8</td>
</tr>
<tr>
<td>rscu-PA</td>
<td>0.84±0.12</td>
<td>34±3.7</td>
<td>81±5.7</td>
</tr>
<tr>
<td>rt-PA</td>
<td>1.1±0.16</td>
<td>25±3.9</td>
<td>77±6.0</td>
</tr>
</tbody>
</table>

Data represent mean±SEM of four experiments each, obtained by fitting the individual thrombolysis data with an exponentially transformed sigmoidal function \( y = \frac{c}{1+e^{-(x-a)/b}} \) (see “Methods”).
proportional relative increase in the number of binding sites for MA-15C5 (up to 10-fold), whereas the number of binding sites for MA-12B3 remained unchanged. The thrombolytic potency of rscu-PA/MA-15C5 was previously found to be ninefold higher than that of rscu-PA in the rabbit jugular vein thrombosis model.\textsuperscript{14} We found that the thrombolytic potencies of rscu-PA/MA-12B3 both in rabbits with a human plasma clot in the jugular vein and in hamsters with an experimental pulmonary embolus consisting of a human plasma clot were comparable to those of rscu-PA/MA-15C5. Thus, despite the sixfold higher clot-binding capacity of MA-12B3 to native clots, its clot-targeting potency was not higher than that of MA-15C5. These data indicate that the clot-targeting potency is not exclusively determined by the affinity and binding capacity of fibrin-specific antibodies for native clots but that it probably is the resultant of several interacting parameters, including alteration of binding during digestion of the clot as well as the extent and rate of initial binding.

To gain some insight in the parameters that determine the extent of clot lysis, its time course was measured by continuous external radioisotope scanning in the hamster model.\textsuperscript{31,32} At doses that induced maximal clot lysis within 90 minutes, lysis with all compounds was still characterized by a lag phase of 18 minutes with rscu-PA/MA-12B3, 28 minutes with rscu-PA/MA-15C5 (p = NS versus rscu-PA/MA-12B3), and 34 minutes with rscu-PA (p = 0.03 versus rscu-PA/MA-12B3). Furthermore, the maximal rates of clot lysis (slope of the time course curve at the inflection point) and the time required to reach maximal clot lysis were very similar at approximately 80 minutes for all compounds. These data indicated that although clot targeting of rscu-PA resulted in a significant reduction of the dose required to obtain maximal clot lysis, clot targeting did not result in a significant increase of the maximal rate of lysis or in a significant reduction of the lag phase before initiation of clot lysis. Because these latter observations were surprising, the thrombolytic parameters of the two rscu-PA/antifibrin conjugates were also compared with those of rt-PA. The thrombolytic potency of rt-PA was sixfold lower than those of the rscu-PA/antifibrin conjugates but sixfold higher than that of rscu-PA. The latter finding is apparently at variance with the experience in patients with myocardial infarction, where it was shown that the therapeutic doses of rt-PA and rscu-PA are very similar.\textsuperscript{35,36} However, it is well known that rodents in general are more resistant to rscu-PA than to rt-PA.\textsuperscript{37,38} The time course of clot lysis with rt-PA, when fitted with the exponentially transformed sigmoidal function as described in “Methods,” revealed a lag phase of 25±3.9 minutes (n = 4), a maximal lysis rate of 1.1±0.16% per minute, and a time to maximal clot lysis of 77±6 minutes. None of these parameters were significantly different from the corresponding parameters for rscu-PA/MA-12B3, rscu-PA/MA-15C5, and rscu-PA and did not change significantly at a fourfold higher dose of rt-PA.

Our data confirm that antibody-targeting increases the thrombolytic potency (percent lysis per unit dose administered) as well as the specific thrombolytic activity (lysis per unit steady-state plasma level) of rscu-PA, resulting in a significant reduction of its dose required for effective clot lysis. Increased specific activities could also be associated with reduced side effects, although this remains to be demonstrated.

In conclusion, the methods and models of the present study allowed the systematic evaluation of the effect of clot targeting on the extent of clot lysis, on the lag phase before the initiation of clot lysis, and on the maximal rate of clot lysis. The results suggest that clot targeting may produce more potent thrombolytic agents but that there may be a ceiling for the maximal achievable speed of clot lysis imposed by intrinsic maximal rates of lysis and minimal lag phases, which are independent of the nature of the plasminogen activator or of clot targeting.

Acknowledgments

We thank I. VanLinthout and E. Brouwers for technical assistance and J. Vangoetsehoven and B. Verheyden for secretarial assistance.

References

20. Tymkewycz PM, Gascoine FS, Gaffney PJ: A monoclonal antibody (MAB) to an A-alpha chain epitope which is exposed only on the surface of fibrin. (abstract) Thromb Haemost 1989;62:478
22. Dederle PJ, Van Keer L, Verstreken M, Collen D: An enzyme-linked immunosorbent assay for urokinase-type plasminogen activator (u-PA) and mutants and chimeras containing the serine protease domain of u-PA. Thromb Haemost 1992;67:95–100
32. Stassen JM, Vanlinhout I, Lijnen HR, Collen D: A hamster pulmonary embolism model for the evaluation of the thrombolytic and pharmacokinetic properties of thrombolytic agents. Fibrinolysis 1990;4:15–21
Thrombolytic profiles of clot-targeted plasminogen activators. Parameters determining potency and initial and maximal rates.

P Holvoet, M Dewerchin, J M Stassen, H R Lijnen, T Tollenaere, P J Gaffney and D Collen

Circulation. 1993;87:1007-1016
doi: 10.1161/01.CIR.87.3.1007

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
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/87/3/1007

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/