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EXPERIMENTAL STUDIES

"Plasminogen Steal" and Clot Lysis

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Although initially developed to reduce the risk of bleeding, second-generation (clot-selective) thrombolytic agents have been found to induce more prompt and frequent recanalization than do nonselective, first-generation agents. To determine whether they do so in part by preserving clot-associated plasminogen, human whole blood clots formed in Chandler tubes were studied. Addition of supratherapeutic concentrations of recombinant tissue-type plasminogen activator (rt-PA) to the media bathing mature clots led to a paradoxic impairment of clot lysis and a concomitant concentration-dependent depletion of clot-associated plasminogen (Western blot analysis). In contrast, supplementation of the plasma with plasminogen (0.27 mg/ml) led to significant conservation of both plasma and clot-associated plasminogen ($p \leq 0.05$, $n = 4$), and prevented the diminution of clot lysis ($p \leq 0.05$; $n = 4$).

Fibrinogen degradation products did not account for the attenuation of lysis with the highest concentrations of rt-PA. In concentrations equivalent to those that were induced by the highest concentrations of rt-PA evaluated, fibrinogen degradation products potentiated rather than inhibited lysis ($p \leq 0.05$, $n = 4$), probably by stimulating rt-PA activity directly. When preformed clots were incubated with plasminogen-depleted plasma plus 1,000 ng/ml rt-PA, the plasminogen content in residual clot declined (9.36 ± 0.46 versus 12.39 ± 0.69 ng/mg clot found in nondepleted plasma; $p \leq 0.05$; $n = 6$). Furthermore, clot lysis was attenuated completely.

Thus, clot lysis induced by activation of plasminogen is dependent on clot-associated plasminogen, which in turn depends on the concentration of plasminogen in plasma. Depletion of both is likely to contribute to the lower frequency and rapidity of coronary recanalization induced with nonclot-selective compared with selective fibrinolytic agents because of the depletion of plasma plasminogen and the plasminogen steal they characteristically induce.

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Agents used to induce fibrinolysis clinically differ with respect to their relative clot or fibrin selectivity. Second-generation agents such as recombinant tissue-type plasminogen activator (rt-PA) (alteplase or Activase) and prourokinase (Saruplase) are more clot selective than are first-generation agents such as streptokinase (Streptase), anisoylated plasminogen activator complexes (APSAC) (anistreplase or Eminase) and urokinase (1–3). Second-generation agents were developed initially to reduce the risk of bleeding. However, they have been found to induce recanalization of coronary arteries more promptly and more frequently than do first-generation agents (4–6). We hypothesized that their enhanced efficacy is a reflection, in part, of a paradoxic diminution of clot lysis seen when plasminemia and concomitant depletion of fibrinogen and plasminogen from blood occur with first-generation fibrinolytic agents—phenomena encountered regularly with their administration (7,8). We postulated that depletion of plasminogen from plasma would result in leaching of clot-bound plasminogen into the plasminogen-depleted fluid phase ("plasminogen steal") (9).

Although changes in plasma plasminogen accompanying fibrinolysis are consistent with this hypothesis, quantitative relations among induced changes in concentrations of plasma plasminogen, clot-associated plasminogen and rate and extent of clot lysis have not yet been characterized. The present study was performed with a second-generation agent to define these relations and to determine whether plasminogen steal is likely to contribute to the differential effects of first- compared with second-generation fibrinolytic agents on the rapidity and frequency of coronary recanalization.

Methods

Experiments were performed in vitro with human recombinant deoxyribonucleic acid (DNA) tissue-type plasminogen activator (rt-PA) produced in Chinese hamster ovary cells (Genentech). Enzyme-linked immunosorbent assay (ELISA) reagents were obtained from American Diagnostica for assay of rt-PA performed as previously described (10). High molecular weight protein standards for use with sodium dodecyl-sulfate polyacrylamide gel (SDS-PAGE) electrophoresis and Western blotting were obtained from BioRad.

Induction of clots in vitro. Informed written consent was obtained from all subjects for blood sampling, and the study was conducted in accordance with the Ethical Guidelines of

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the Human Studies Committee of the Washington University School of Medicine.

Human blood clots were formed in Chandler tubes as previously described (9,11) after collection of samples from the anticubital vein in normal volunteers with a 19-gauge needle and a polypropylene syringe. Blood was transferred quickly to a 27-cm long Tygon tube (internal diameter = 0.125 in. [0.318 cm]; outer diameter = 0.188 in. [0.475 cm], 1 ml blood per tube; Fisher Scientific) that was connected at both ends with a 1 cm collar of Tygon tubing (internal diameter 0.188 in. (0.475 cm); outer diameter 0.312 in. (0.792 cm). After addition of 3.5 μg of fibrinogen (Kabi Vitrum), the tubes were rotated on a tube rotator (Scientific Equipment Company) for 60 min at an angle of 60° at 30 rpm, simulating blood flows of approximately 30 ml/min. Clot formation was complete within 1 h as documented previously (12).

Effects of depletion of plasminogen from the fluid phase. Venous blood was drawn into sodium citrate (3.8%) and centrifuged at 1,150 × *g* for 10 min to obtain plasma from normal volunteers who had not taken any medication including aspirin and nonsteroid anti-inflammatory agents for at least 3 weeks. Goat anti-human plasminogen (Sigma P-5276) was added to a final concentration of 20 mg/ml. An equal volume of plasma was placed in another tube that contained no antibody. After vigorous shaking of the samples in both tubes overnight on a rotator at 4°C, protein G was added (1:10 volume/volume) to precipitate the plasminogen. After further incubation and rotation for 2 h at room temperature, the plasma was centrifuged at 1,100 × *g* for 10 min at 4°C. The supernatant fraction was removed and assayed for residual plasminogen with a chromogenic substrate (S-2251) and reagents from Kabi Vitrum. The concentration of plasminogen in the depleted plasma that had been treated with antibody was only 5% of that in autologous control plasma (n = 2).

Clots that had been formed in Chandler tubes were transferred into tubes with either normal or plasminogen-depleted plasma and incubated while rotating at 37°C for an additional 60 min. Subsequently, the concentration of plasmin that had been generated in the fluid phase of normal or plasminogen-depleted plasma bathing the clots was assayed with another kinetic chromogenic substrate assay (Spectrozyme-PL, American Diagnostica).

Effects on clot lysis of supplementation of blood with fibrinogen degradation products. To determine whether generation of high concentrations of fibrinogen degradation products accompanying generation of plasmin may account for the decreased rate and extent of clot lysis attributed to plasminogen steal by competing with the binding of activator to fibrin, we performed experiments with the addition of high concentrations of fibrinogen degradation products (50 μg/ml of whole blood). Fibrinogen degradation products were prepared by incubating plasmin (Kabi Vitrum) with exogenous fibrinogen (Kabi Vitrum) for 15 min at 37°C and were subsequently assayed by the staphylococcal clumping method (Sigma Diagnostic Kit No. 850). Clots were allowed to mature for 1 h before small volumes of a concentrated solution of fibrinogen degradation products or saline solution were added to the whole blood. After a 5-min incubation with either the fibrinogen degradation products or saline solution, selected concentrations of rt-PA (1,000 to 8,000 ng/ml) were added to the medium. The extent of clot lysis was assayed gravimetrically on the basis of the percent reduction of dry weight of treated clots compared with the weight of untreated clots from the same blood samples.

Extraction of clot-associated plasminogen. Each clot was removed from the Chandler tube at the end of the experiment and washed extensively over a polyethylene mesh filter (Spectrum Medical Industry, No. 146386) with saline solution containing Tween-20 (0.05% volume/volume). After the wet weight of clots had been measured, the clots were homogenized manually at 4°C in a Dual glass homogenizer in 100 μl of sodium chloride (NaCl), 20 m*M*; Tris, 10 m*M*; ethylenediaminetetroacetic acid (EDTA), 1 m*M*; epsilon-amino caproic acid (ε-ACA), 15 m*M*; Triton X-100, 0.5%, and aprotinin, 400 KIU/ml at pH 8. (d-Phenylalanyl)-L-prolyl-L-arginine chloromethyl ketone (PPACK) (purchased from Calbiochem) was added to the homogenate to yield a final concentration of 2 μ*M*. The homogenizer was rinsed with 50 μl of the same buffer, the rinse was combined with the homogenate, and both were centrifuged in centricon-3 microconcentrator tubes (Amicon). The microconcentrators were presoaked for 24 h with 2 ml of phosphate-buffered saline solution containing bovine serum albumin, 5 mg/ml, and bovine gamma globulin, 2.5 ng/ml. Before addition of the homogenate, the phosphate-buffered saline solution was discarded and the microconcentrator tube was centrifuged for 2 min at 1,000 × *g* at 4°C in a Sorvall RC-5 centrifuge.

Homogenates were centrifuged in the microconcentrator tubes at 5,000 × *g* at 4°C for 1 h. The filtrate was discarded, and solvent of the retentate was supplemented three times with 9 volumes of sodium phosphate buffer containing 400 KIU/ml aprotonin at pH 8 per 1 volume of retentate, which was centrifuged at 5,000 × *g* at 4°C until the volume of the retentate was approximately 40 μl (usually overnight). The final retentate was centrifuged at 1,000 × *g* for 2 min. The filter was washed with 20 μl of the same buffer, and the 60-μl final combined volume was stored at −70°C until Western blot analysis was performed to quantify plasminogen that had been extracted from the clots.

Assay of activatable plasminogen and of plasmin. Concentrations of activatable plasminogen were determined by incubating 50 μl of plasma with 50 μl of 0.166 *M* hydrogen chloride (HCl) at room temperature for 15 min, neutralizing the sample with 50 μl of 0.166 *M* sodium hydroxide to inactivate inhibitors, diluting with 1.0 ml of 50 m*M* Tris-HCl, 12 m*M* NaCl, pH 7.4, adding streptokinase (1,000 U in 100 μl water, Sigma) to 200 μl of diluted sample in a spectrophotometer cuvette at 37°C, adding 700 μl of buffer containing the chromogenic substrate, S-2251 (final concentration = 0.8 m*M*), and measuring A_{405} as a function of time. Standard curves were obtained with several dilutions of pooled (n = 10 donors), citrated, normal human plasma.

Values were expressed as a percent of values in untreated control samples.

For assay of plasmin, a second chromogenic substrate, Spectrozyme PL (ADI), was used. Samples were collected into 2-ml EDTA-vacutainer tubes (Becton Dickinson) containing a final concentration of 2 μM of PPACK and were assayed immediately. Standard curves were obtained with plasmin (Sigma) by measuring A_{405} as a function of time. Standard curves obtained with and without PPACK did not differ, indicating that PPACK had no effect on measurable plasmin activity under the conditions employed.

Quantification of plasminogen. For quantitation of clot-bound plasminogen, Western blotting was performed. After clots had been homogenized and extracted, 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed under nonreducing conditions with samples and standards (3.13- to 100-μg/ml human glu-plasminogen, ADI No. 410) in slab gels according to the method of Laemmli (13). Sequential proteins in the gel were transferred electrostatically to 0.45 μm nitrocellulose (Schleicher and Schuell) at 4°C (100 V, 0.3 A for 60 min). The gel and nitrocellulose sandwich was removed and blocked overnight with 50 ml of buffer containing Tris-HCl, 50 mM; NaCl, 150 mM; and 2% bovine serum albumin (Buffer A), pH 7.5, at 4°C with constant agitation. Iodine-125 goat antimouse immunoglobulin G (1.3×10^6 cpm/ml) was prepared with goat antimouse immunoglobulin G (Sigma, M-8890) and carrier-free sodium iodide-125 (Amersham) by the chloramine-T method. A monoclonal antibody against human glu-plasminogen, which cross reacts with plasmin only minimally (14) was used to detect plasminogen (ADI no. 3641). The nitrocellulose was incubated for 60 min with 100 ng/ml of the antibody with constant agitation at 37°C and washed four times at room temperature (10 min/wash) with 20 ml of buffer containing Tris-HCl, 50 mM; NaCl, 150 mM; 0.2% of sodium dodecyl sulfate, 0.5% bovine serum albumin and 0.5% of Triton X-100 (Buffer B); it was then washed once with 20 ml of Buffer A. The nitrocellulose was then incubated for 60 min with 20 ml of I-125 goat antimouse immunoglobulin G (1.3×10^6 cpm/ml, specific activity = 0.26×10^6 cpm/μg) at 37°C with agitation. The nitrocellulose was washed again four times with 20 ml of Buffer B for 10 min and once more with 20 ml of distilled water with agitation for 10 min. Autoradiograms were developed with Kodak Diagnostic Filters (X-OMAT, XAR-5) and Cronex intensifier screens at −70°C for 18 to 24 h. Areas corresponding to labeled plasminogen bands were cut out, and radioactivity was quantified by gamma counting. The recovery of plasminogen as judged from results with added standards was 93% (n = 4).

Effects on clot lysis of supplementation of medium bathing clots with plasminogen. To determine whether supplementation with plasminogen of medium bathing clots attenuated effects attributed to plasminogen steal (i.e., the paradoxically decreased clot lysis observed with sustained, high concentrations of rt-PA), clots were formed by addition of 3.5 μg of fibrinogen to 1 ml of whole blood and rotated for 1 h at 37°C. Either rt-PA (final concentration 4,000 ng/ml) or phosphate-buffered saline solution was added in equal volume, and the tubes were rotated at 37°C for an additional 30 min. Either human glu-plasminogen (final concentration 0.27 mg/ml) or phosphate-buffered saline solution in equal volume was added after 6 min and again after 16 min of incubation. When incubation was complete, the fibrin-rich head of the clot was removed (15) and placed into a dual glass homogenizer in 100 μl of NaCl, 20 mM; Tris, 10 mM; EDTA, 1 mM; ε-ACA, 15 mM; 0.5% Triton X-100 and aprotinin, 400 KIU/ml, at pH 8. An additional 50 μl of buffer was used to rinse the sides of the homogenizer. PPACK was added to the homogenate (final concentration 2 μM), and the combined homogenate and rinse were centrifuged in microconcentrator tubes at $5,000 \times g$ for 60 min at 4°C. The volume in the microconcentrator was brought to 450 μl with a sodium phosphate buffer containing aprotinin, 400 kIU/ml. The retentate was centrifuged again for 2 h at $5,000 \times g$ at 4°C, the volume was adjusted to 2 ml with sodium phosphate buffer supplemented with aprotinin (400 kIU/ml), and the mixture was centrifuged overnight at $5,000 \times g$ at 4°C to yield a final volume of approximately 40 μl. The filters were rinsed with 20 μl of the sodium phosphate with aprotinin buffer and the rinses were combined with the retentates. The combined extract was stored at −70°C before performance of Western blotting for quantification of extracted plasminogen.

Statistics. Results were expressed as mean values ± SD. Mean values were compared by Student *t* tests. Significance was defined as $p \leq 0.05$.

Results

Depletion of plasminogen in clots. The plasminogen content in residual clots was determined by Western blot analysis and expressed as both ng/mg of clot and the percent of that in control clots. One hundred percent plasminogen is equivalent to 64.7 ng/mg of plasminogen (53.4, 76, n = 2). Figure 1 shows that clot-associated plasminogen declined after 30 min of incubation with sustained, high concentrations of rt-PA. The highest concentrations of rt-PA resulted in the greatest diminution of plasminogen in residual clots.

Effects of supplementation with plasminogen. To determine whether the depletion of plasminogen from clots was secondary to depletion of plasma plasminogen and responsible for the paradoxical diminution of clot lysis with high concentrations of rt-PA (plasminogen steal), additional clots were exposed to the same concentrations of rt-PA (4,000 ng/ml) in plasma supplemented with equal volumes of solutions of plasminogen (3 μM; n = 4) or buffer. In tubes without supplemental plasminogen, the percent of clot lysis that occurred was only 31.8 ± 16% (n = 4). In contrast, in tubes with supplemental plasminogen, the percent of lysis was increased to 73.9 ± 10% (n = 4). Figure 2A shows the amount of clot-associated plasminogen expressed as a percent of that in control clots incubated without either rt-PA or supplemental plasminogen. Conservation of clot-associated

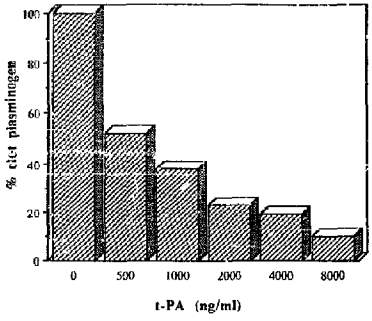


Figure 1. Human clots were formed from whole blood in Chandler tubes for 60 min at 37°C and incubated for an additional 30 min with added recombinant tissue-type plasminogen activator (t-PA). Depletion of clot-associated plasminogen was greatest with increasing concentration of t-PA (n = 2 for each set of conditions).

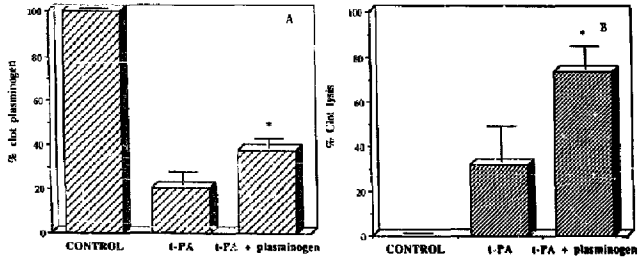
plasminogen induced with supplementation of the fluid phase with 3 μM of plasminogen is evident. Figure 2B shows the potentiation of lysis of clots incubated with rt-PA by supplemental plasminogen ($p < 0.05$) compared with that of clots incubated with rt-PA alone.

Effects of fibrin degradation products on clot lysis induced by rt-PA. To determine whether fibrinogen degradation products generated by high concentrations of rt-PA could account for apparent plasminogen steal and paradoxic attenuation of clot lysis by competing for binding of plasminogen activator to fibrin, clots formed in Chandler tubes were exposed to rt-PA in the presence or absence of exogenous fibrinogen degradation products (50 μg/ml). Preliminary results showed that the maximal concentration of fibrinogen degradation products, generated in 90-min incubations with 8,000 ng/ml of rt-PA, was <50 μg/ml. Fibrinogen degradation products did not attenuate lysis with any of the concentrations of rt-PA used (Fig. 3). In fact, lysis was potentiated significantly at both 4,000 and 8,000 ng/ml of rt-PA ($p \leq 0.05$; n = 8), consistent with some potentiation of activation of plasminogen by the fibrinogen degradation products themselves (16).

Effects of depletion of plasminogen induced without rt-PA on clot-associated plasminogen and subsequent clot lysis with rt-PA. After formation of whole blood clots in Chandler tubes for 60 min, some clots were transferred to plasminogen-depleted plasma and incubated for 30 min with rt-PA (1,000 ng/ml). Control clots were placed in normal plasma containing physiologic concentrations of plasminogen with the same concentration of rt-PA. The resulting lysis and residual clot-associated plasminogen were determined gravimetrically and by Western blotting.

The concentration of free plasmin remaining in the fluid phase after the formation of the clot in the 1st 30 min of incubation was similar for both sets of clots (0.028 ± 0.003 CU/ml for those incubated in plasminogen-depleted plasma compared with 0.027 ± 0.003 CU/ml for those incubated in normal plasma [n = 4]), indicating similar contributions of fluid-phase free plasmin in both sets of conditions. Lysis of clots incubated in the plasminogen-depleted plasma with 1,000 ng/ml rt-PA was attenuated compared with that of clots incubated in normal plasma supplemented with rt-PA ($p \leq 0.05$) (Fig. 4A). This concentration of rt-PA was chosen to determine whether lower concentrations of rt-PA can induce plasminogen steal when fluid-phase plasminogen is depleted by an alternative method. Clot lysis was even greater when preformed clots were incubated in whole blood supplemented with rt-PA

Figure 2. Clots were formed in whole blood Chandler tubes with and without supplementation with plasminogen. In tubes that were supplemented with plasminogen, clot-associated plasminogen (A) as a percent of that in control clots was slightly but significantly increased (asterisk, $p < 0.05$; n = 4). Clot lysis (B) was significantly increased in samples supplemented with plasminogen compared with those supplemented with recombinant tissue-type plasminogen activator (t-PA) alone (asterisk, $p < 0.05$; n = 4).



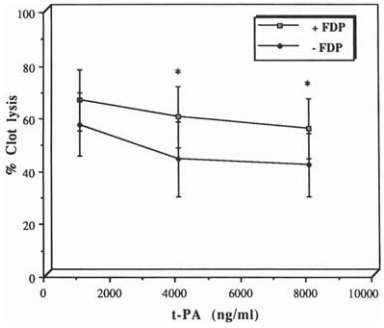


Figure 3. Fibrinogen degradation products (FDP) (50 μg/ml) were added to some samples with recombinant tissue-type plasminogen activator (t-PA) (1,000 to 8,000 ng/ml) to determine whether the generation of fibrinogen degradation products by t-PA could be responsible for the diminution of clot lysis seen with high concentrations of plasminogen activators. At high concentrations (4,000 and 8,000 ng/ml) of t-PA, lysis was enhanced rather than attenuated (n = 8). Statistical significance ($p \leq 0.05$) of differences between lysis with and without fibrinogen degradation products is denoted by asterisks.

(n = 8) than when they were incubated in plasma. Clots formed in whole blood and exposed to no exogenous rt-PA contained 26.5 ± 1.78 ng of plasminogen per mg of clot (n = 2). The concentration of clot-associated plasminogen was significantly lower ($p < 0.01$) when clots were incubated subsequently in plasminogen-depleted plasma (9.36 ± 0.46 ng/mg; n = 6) rather than in normal plasma (12.39 ± 0.69; n = 6) (Fig. 4B).

Discussion

The present study was performed to explore our recent hypothesis that plasminogen steal can account for a limitation of clot lysis with nonselective plasminogen activators because of depletion of plasminogen from whole blood in vivo. Results obtained show that the paradoxic diminution of clot lysis seen with supraphamacologic, high concentrations of rt-PA is associated not only with depletion of plasminogen from the fluid phase, but also with depletion of clot-associated plasminogen, reflecting an equilibrium between the two with a consequent reduction of the availability of clot-associated substrate (plasminogen) for activation.

Effect of depletion of plasminogen on lysis. Confirmation of a causal connection between depletion of plasminogen and attenuation of lysis was obtained in experiments in which whole blood clots were incubated in plasminogen-depleted plasma supplemented with rt-PA. Clot lysis was attenuated compared with lysis of clots incubated with rt-PA in plasma with physiologic concentrations of plasminogen despite the presence of similar concentrations of free plasmin in the fluid phase under both sets of conditions. These results indicate that the attenuation of clot lysis is a result of depletion of clot-associated plasminogen rather than depletion of plasminogen in the fluid phase itself, whether or not plasmin is present in the fluid phase.

As was the case with clots incubated with the highest concentrations of rt-PA, clot-associated plasminogen was decreased in clots incubated with lower concentrations of rt-PA in plasminogen-deficient plasma. In addition, lysis was attenuated. These observations corroborate the diminution of plasminogen in the fluid phase as a determinant, regardless of how it is induced, of both depletion of clot-associated plasminogen and attenuation of clot lysis. The greater induction of lysis of clots incubated in whole blood than of clots incubated in plasma with physiologic concentrations of plasminogen may be attributable to contributions of red blood cells and platelets to lysis of clots induced by plasminogen activators. Cellular elements such as platelets can facilitate activation of plasminogen by providing a surface on which plasminogen and rt-PA can bind and interact (17,18).

Clinical implications of plasminemia. Relatively clot-selective plasminogen activators were developed on the basis of the premise that induction of clot lysis without the plasminemia associated with nonclot-selective drugs would

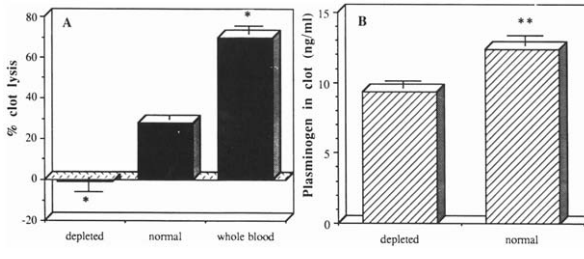


Figure 4. Preformed clots were incubated with or without recombinant tissue-type plasminogen activator (t-PA) (1,000 ng/ml) in plasminogen-depleted or normal plasma. Clot lysis (A) was virtually abolished in the plasminogen-depleted plasma ($p \leq 0.05$; n = 8) and clot-associated plasminogen was depleted ($p \leq 0.01$; n = 6) compared with that seen in clots exposed to t-PA in normal plasma (B). Statistical significance is denoted by one ($p \leq 0.05$) or two ($p \leq 0.01$) asterisks.

reduce the risk of bleeding. Surprisingly, however, compared with first-generation agents, these newer agents were found to induce a more frequent and more rapid recanalization of infarct-related arteries (2,6,19). These phenomena may be explained in part by recently characterized plasmin-mediated prothrombotic effects of plasminogen activators (20,21) that can shift the balance between dynamic thrombolytic and thrombotic processes toward thrombosis, with consequently delayed recanalization and decreased frequency of recanalization with first-generation agents. High concentrations of plasmin can activate platelets in vitro and in vivo (22–24), an effect that may contribute to potentiation of thrombosis by first-generation agents as well.

As shown in the present study, induction of plasminemia may exert adverse effects by additional mechanisms. Thus, marked activation of circulating plasminogen with a corresponding diminution of the concentration of plasminogen can induce plasminogen steal with consequent attenuation of clot lysis. Depletion of plasminogen is, of course, more marked with first- than with second-generation plasminogen activators because of the relative clot selectivity of second-generation agents.

Effect of fibrinogen degradation products. The paradoxic reduction in lysis seen with plasminogen steal in the present study is not attributable to high concentrations of fibrinogen degradation products that could hypothetically compete with fibrin for binding of the plasminogen activator. This addition of exogenous fibrinogen degradation products did not diminish lysis. In fact, the opposite occurred—that is, potentiation of lysis—an effect that may reflect binding of both rt-PA and plasminogen to the E-domain of fibrinogen degradation products and enhancement of activation of plasminogen (16). Obviously, plasmin in the fluid phase could contribute to lysis directly if $alpha_2$-antiplasmin was already consumed. Conversely, the depletion of plasminogen accompanying generation of plasmin could reduce lysis by inducing plasminogen steal. The balance is probably governed by the extent to which $alpha_2$-antiplasmin persists in the fluid phase. Regardless, generation of fibrinogen degradation products does not account for the attenuation of clot lysis seen under conditions in which plasminogen steal occurs.

Conclusions. Our results are consistent with the hypothesis that induction of thrombolysis with clot-selective agents facilitates recanalization by avoiding marked plasminemia and the subsequent attenuation of lysis accompanying plasminogen steal.

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