Comparison of streptokinase, urokinase, and recombinant tissue plasminogen activator in an in vitro model of venous thrombolysis

Kenneth Ouriel, MD, Ethan L. Welch, MD, Cynthia K. Shortell, MD, Kevin Geary, MD, William M. Fiore, MD, and **Cathy Cimino, BA,** *Rochester, N.Y.*

Purpose: **Presumed differences in the thrombolytic activity and fibrinolytic specificity of the three commonly used thrombolytic agents, streptokinase, urokinase, and recombinant tissue plasminogen activator (rt-PA), are based on clinical study results, where variability renders meaningful comparisons difficult. An in vitro model of catheter-directed venous thrombolysis was used to compare the three agents.**

Methods: **Retracted iodine 125–radiolabeled clots that simulate those observed in the venous system were infused with thrombolytic agents at doses analogous to those used clinically. Perfusion with heparinized, whole human blood was undertaken for 60 minutes, measuring the efficacy of thrombolysis through serial quantification of radio tracer released into the circuit. Fibrinolytic specificity was determined by following decrements in perfusate fibrinogen concentration.**

Results: **Streptokinase was the agent associated with the slowest rate of clot lysis ($p = 0.01$ vs urokinase and rt-PA). Urokinase was associated with an intermediate rate of lysis but appeared to be the agent with the greatest degree of fibrinolytic specificity ($p = 0.02$ vs streptokinase, $p = 0.05$ vs rt-PA). Although rt-PA was associated with improved efficacy early in the perfusions, the differences between rt-PA and urokinase dissipated after 30 minutes.**

Conclusions: **These laboratory observations suggest that urokinase may be the most appropriate agent for catheter-directed venous thrombolysis, offering an advantageous compromise between fibrinolytic specificity and thrombolytic speed. (J VASC SURG 1995;22:593-7.)**

Administration of thrombolytic agents into vascular thrombi has become accepted as a modality to recanalize occluded arteries[1-5] and veins.[6-9] At present, three commonly used thrombolytic agents exist: streptokinase, urokinase, and recombinant tissue plasminogen activator (rt-PA). Three measures by which these agents can be compared are cost, lytic speed, and fibrinolytic specificity. The agents clearly differ with regard to cost, and they may also differ with regard to rapidity of thrombolysis and fibrinolytic specificity.[10,11] To date, there has been relatively scant investigation comparing the available thrombolytic agents in an objective, experimentally sound manner. The vast number of confounding variables such as duration of the occlusion, anatomic location of the occlusion, and the mass of the thrombus makes the performance of a clinical study impossible without very large numbers of patients.

We have developed an in vitro experimental model that closely resembles the clinical setting.[12] A rational comparison of the available thrombolytic agents can be more easily accomplished in this laboratory setting, controlling for such variables as the size and consistency of the thrombus and the hemodynamic conditions present in the occluded conduit during recanalization. Using this in vitro

From the University of Rochester, Department of Surgery, Section of Vascular Surgery, Rochester.

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Reprint requests: Kenneth Ouriel, MD, Department of Surgery, The University of Rochester, 601 Elmwood Ave., Rochester, NY 14642.

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model of thrombolysis, we have compared streptokinase, urokinase and rt-PA with regard to speed of thrombolysis and fibrinolytic specificity.

MATERIAL AND METHODS

Material. Streptokinase (KabiVitrum AB, Stockholm, Sweden), urokinase (Abbott Laboratories, North Chicago, Ill.), and rt-PA (Genentech, South San Francisco, Calif.) were reconstituted in 5% dextrose to achieve final concentrations of 150 U, 5000 IU, and 80 μg per ml. Porcine sodium heparin (0.75 U/ml, Elkins-Sinn, Cherry Hill, N.J.) was used to anticoagulate the whole blood perfusate, raising the activated clotting time from 120 ± 3 seconds to 268 ± 7 seconds. Topical bovine thrombin (Armour Pharmaceutical Co, Kankakee, Ill.) and iodine 125–labeled fibrinogen (0.813 Ci/ml; ICN Biomedicals, Irvine, Calif.) were used to form thrombus. Nonthrombogenic plastic tubing and connectors were used in the perfusion circuit to minimize activation of platelets and the clotting cascade.[12]

Clot formation. Phlebotomy was performed on 37 healthy volunteers who had not recently received antiplatelet agents or oral contraceptive medications, drawing a total of 80 ml of blood into syringes preloaded with 60 U heparin (0.75 U/ml final heparin concentration). Immediately, 10 ml heparinized blood was combined with 500 IU thrombin and 1.0 μCi ^{125}I-fibrinogen in a plastic syringe. The remaining 70 ml of heparinized blood was saved for use as the perfusate.

Once formed, the clots were incubated for 90 minutes at 37° C and allowed to retract, decanting the serum from the syringe. The clots were then removed from the syringe, cut into strips, and gently packed into 5 cm long, 5 mm internal diameter polytetrafluoroethylene segments. The initial amount of clot was then quantitated by measuring the ^{125}I activity of the segment in a gamma counter. The efficiency of the gamma counting protocol was 97.14% to 0.4%.

Perfusions. The perfusion model was fully described in a previous publication.[12] Thirty-seven perfusions were performed in this study, representing one perfusion for each volunteer donor. Clots were infused with streptokinase (12 perfusions, 10 U/min), urokinase (15 perfusions, 333 IU/min), or rt-PA (10 perfusions, 5.3 mcg/min) at a rate of 0.067 ml per minute for 60 minutes with use of a volumetric infusion pump (Imed Corp, San Diego, Calif.). The infusion catheter was threaded into the thrombus with the single end-hole located in the proximal portion (1.0 cm) of clot. The thrombolytic infusion rates and doses were analogous to those used clinically, scaling the clinical parameters down by a factor of one eleventh to account for differences in the mass of thrombus. Calculations were based on an average thrombus mass of 11 grams in a 15 cm long, 1 cm diameter iliofemoral venous segment, versus 1 gm in the 5 cm long, 5 mm diameter conduit.

Phasic perfusate flow was generated with a peristaltic pump (Manostat, Varistaltic, Rochester, N.Y.) by use of a bypass circuit around the clot-containing segment to simulate collateral flow around an occluded vein. The temperature of the system was maintained at 37° C, enclosing the system in a lead-lined, thermostatically controlled, heated box. Total flow through the perfusion circuit was controlled at 40 ml/min. Flow through the polytetrafluoroethylene segments ranged from 0% to 36% ± 2% of total flow in the circuit as clot dissolution progressed, corresponding to shear rates of 0 to 150 sec^{-1} and analogous to the hemodynamic conditions observed in large veins.[13]

Fibrinolytic specificity was assessed by measuring the decrement in perfusate fibrinogen over time, reasoning that the most fibrin-specific agent would be the agent with the least propensity for systemic fibrinogen degradation. Fibrinogen concentrations were assayed spectrophotometrically by use of the method of Clauss,[14] measuring fibrinogen in serial aliquots of the perfusate, drawn into tubes preloaded with aprotinin (Sigma Chemical Co., St. Louis, Mo.) to arrest ongoing fibrinogenolysis.

Fibrinogen disappearance in the perfusate occurred almost exclusively as a result of the infusion of thrombolytic agents and fibrinogenolysis. The conversion of fibrinogen to fibrin in the circuit was minimal as a result of excess heparin in the perfusate and complete inhibition of thrombin. Fibrinopeptide A levels, an index of fibrinogen disappearance through thrombogenesis, averaged less than 20 ng/ml in 120-minute control perfusions performed in the absence of thrombolytic agent.

Statistics. Data were analyzed over time with use of Student's *t* test and analysis of variance techniques. Significance was assumed when the two-tailed *p* value was less than 0.05. Data are expressed as mean + SEM.

RESULTS

Thrombolytic efficacy. Streptokinase was associated with the slowest rate of thrombolysis (Table I). At 60 minutes, only 27% ± 8% of the thrombus was dissolved ($p = 0.01$). By contrast, urokinase and

Table I. Thrombolytic efficacy (percent clot dissolution) of streptokinase, urokinase, and rt-PA at 15-minute intervals over a 60-minute infusion period

	No.	*15 min.*	*30 min.*	*45 min.*	*60 min.*
Streptokinase	12	8% ± 2%	10% ± 3%	19% ± 5%	27% ± 8%
Urokinase	15	12% ± 3%	31% ± 5%	59% ± 7%	64% ± 9%
rt-PA	10	24% ± 7%	54% ± 8%	71% ± 10%	66% ± 11%

rt-PA perfusions dissolved 64% ± 9% and 66% ± 11% of thrombi, respectively, after 60 minutes of infusion. Although the final amount of thrombus dissolved was equal in the urokinase and rt-PA perfusions, rt-PA was associated with a more rapid rate of thrombolysis early on, averaging 24% ± 7% versus 12% ± 3% in the urokinase perfusions after 15 minutes of infusion ($p = 0.07$). The urokinase/rt-PA differences persisted to 30 minutes ($p = 0.01$) but had dissipated by 45 minutes of perfusion ($p = 0.36$). Thus within the context of the doses used clinically, streptokinase was the slowest agent, urokinase was intermediate, and rt-PA was the agent associated with the most rapid rate of thrombolysis.

Fibrinolytic specificity. Fibrinogen degradation, as an inverse index of fibrinolytic specificity, was similar in the three groups at 30 minutes of infusion (Table II); however, streptokinase was associated a twofold increase in fibrinogen degradation when compared with urokinase (25% ± 5% versus 12% ± 3% at 30 minutes, $p = 0.01$). After 60 minutes of perfusion, fibrinogen degradation was lowest in the urokinase group, averaging 33% ± 5% after 60 minutes of infusion ($p = 0.02$ vs streptokinase, $p = 0.05$ vs rt-PA). Streptokinase and rt-PA were associated with equal rates of fibrinogen degradation, averaging 55% ± 9% and 49% ± 7%, respectively.

Cost analysis. The clinical cost of a vial of streptokinase at the time of study was $297 for 1.5 million units, versus $256 for 250,000 IU urokinase and $2217 for 100 mg rt-PA. Expressing the efficacy of thrombolysis as a function of the cost of the pharmaceutical agent, streptokinase was the most cost-efficient agent, followed by urokinase and rt-PA (Table III).

Table II. Degree of fibrinogen degradation associated with streptokinase, urokinase, and rt-PA expressing fibrinogen degradation as present of baseline concentration

	Fibrinogen degradation	
	30 min.	*60 min.*
Streptokinase	25% ± 5%	55% ± 9%
Urokinase	12% ± 3%	33% ± 5%
rt-PA	18% ± 4%	49% ± 7%

DISCUSSION

Plasmin, a proteolytic molecule with 790 amino acid residues and a molecular weight of 88,000 d, is the active agent in thrombolysis.[15] Fibrin-bound plasmin rapidly degrades insoluble fibrin to the transient intermediate degradation products fractions X and Y and the soluble end-products fractions D and E.[16] This process can restore patency to occluded vascular segments, and the efficacy of the clinically used thrombolytic agents streptokinase, urokinase, and rt-PA results from their relative abilities to catalyze the conversion of fibrin-bound plasminogen to plasmin.[17]

The proteolytic activity of plasmin, however, is not specific for pathologic fibrin thrombi.[18] Clearly, plasmin generation within the desirable fibrin plugs at sites of vascular disintegrity is associated with untoward hemorrhagic side effects. Although some investigators suggested that one thrombolytic agent may have benefits over another with regard to lysis of pathologic thrombi versus fibrin plugs,[11] the structural similarity of the two types of thrombi made this contention ludicrous.[19] Improvements in the safety of thrombolytic regimens are more likely to be achieved with modifications in the method of administration rather than through the use of novel plasminogen activators or through efforts to increase the concentration of lytic agent within the thrombus compared with the concentration in the systemic plasma milieu.[1,3,5] This realization, along with the difficulty in achieving any degree of penetration of lytic agent into large peripheral thrombi,[8,20] has fostered the abandonment of systemic thrombolytic administration in favor of catheter-directed approaches in both the arterial[1,2,21] and venous setting.[7,22,23]

This laboratory investigation documented thrombolytic efficacy and fibrinolytic specificity of the three commonly used thrombolytic agents, streptokinase,

Table III. Relative costs and cost-efficiency of streptokinase, urokinase, and rt-PA.* Relative lysis is based on the percent clot lysis after 60 minutes, indexed to streptokinase as 100. Cost-efficiency is defined as the relative lysis per dollar expended.

	Cost/hr treatment†	*Relative lysis*	*Cost-efficiency*
Streptokinase	$37	100	2.70
Urokinase	$246	237	0.96
rt-PA	$277	244	0.88

*Relative lysis is based on the percent clot lysis after 60 minutes, indexed to streptokinase as 100. Cost-efficiency is defined as the relative lysis per dollar expended.
†Hospital costs, 1995 USD, assumes that a minimum of one vial of agent will be needed to generate an eight hour bag for administration.

urokinase, and rt-PA. Previous clinical[24] and laboratory investigations have suggested that streptokinase is the agent associated with the slowest rate of clot dissolution.[15] Our investigation has corroborated these findings in a model that closely resembles the clinical paradigm associated with catheter-directed venous thrombolysis. Other investigations[24] have suggested that rt-PA is the agent associated with the most rapid rate of thrombolysis. This study confirmed an advantage of rt-PA over both streptokinase and urokinase with respect to thrombolytic rate during the early perfusion period. This benefit, however, did not persist, with equal rates of clot lysis between urokinase and rt-PA during the latter period of perfusion. This later finding is consistent with the observations of Meyerovitz et al.,[25] in a randomized clinical study comparing rt-PA and urokinase. More recently, the STILE trial[26] did not find significant differences between rt-PA and urokinase in patients with peripheral arterial occlusion. The somewhat higher cost of rt-PA does not therefore appear to associated with sufficient lytic efficacy to justify its replacement of urokinase as the agent of choice in peripheral arterial or venous thrombolysis.

rt-PA has been shown experimentally to possess a higher affinity for fibrin than either streptokinase or urokinase,[27] presumably as a result of the kringle domain structures of rt-PA, pronglike projections homologous to similar structures in plasminogen.[28] rt-PA was expected to be a "fibrin-specific" agent, producing thrombolysis without significant plasminemia, fibrinogen breakdown, and a systemic "lytic state." Clinical studies, however, have demonstrated that rt-PA is only relatively specific for fibrin,[24] and its use is associated with significant decrements in plasma fibrinogen and an incidence of distant bleeding complications paralleling that of the other agents.[29] The laboratory data in this study are consistent with a significant degree of systemic fibrinogenolysis associated with rt-PA. It is possible that the relatively large doses of rt-PA necessary to achieve rapid thrombolysis overwhelm the thrombolytic system and result in significant free-plasmin generation and subsequent systemic fibrinogen degradation.

In summary, our laboratory findings confirm that streptokinase in the doses used clinically is the least efficient thrombolytic agent, with a relatively slow rate of thrombolysis and with a significant degree of fibrinogenolysis. Urokinase is intermediate in its rate of thrombolysis, inferior to rt-PA initially, but equal to rt-PA with continued administration. rt-PA appears to offer little advantage over urokinase with respect to fibrinolytic specificity. These observations, taken in conjunction with economic considerations, suggest that urokinase may be the most appropriate agent in catheter-directed venous thrombolysis.

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