A comparison of the thrombolytic and hemorrhagic effects of tissue-type plasminogen activator and streptokinase in rabbits

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ABSTRACT  Tissue-type plasminogen activator (t-PA) is a promising thrombolytic agent because it can produce thrombolysis without inducing a plasma proteolytic state. It is uncertain if this potentially important feature renders t-PA less hemorrhagic than other plasminogen activators. We have compared the hemorrhagic and thrombolytic effects of t-PA and streptokinase in rabbits. Streptokinase, 4000 U/kg/hr over 4 hr, failed to produce significant thrombolysis and 8000 U/kg/hr streptokinase over 4 hr produced only 28 ± 6% thrombolysis. Both streptokinase regimens were associated with a plasmin-mediated plasma proteolytic state and both streptokinase regimens produced a significant increase in hemorrhage that was evident within 15 min of beginning the infusion and was progressive over the 4 hr of drug administration. In contrast, t-PA in a dose of 7500 U/kg/hr produced 35 ± 6% thrombolysis, but it did not produce a plasmin-mediated plasma proteolytic state or a significant increase in hemorrhage over the 4 hr of infusion. t-PA in a dose of 15,000 U/kg/hr produced 85 ± 4% thrombolysis but was associated with a plasmin-mediated proteolytic state and produced significant bleeding which, in contrast to streptokinase-induced bleeding, was delayed in onset. Therefore, t-PA induced less hemorrhage than streptokinase at doses that produced more effective thrombolysis. Bleeding with both thrombolytic agents was associated with a plasmin-mediated proteolytic state.


THROMBOLYTIC THERAPY with streptokinase and urokinase is gaining increasing acceptance as a treatment for venous thrombosis and pulmonary embolism and is presently being reevaluated for the treatment of acute myocardial infarction. The major side effect of thrombolytic therapy is hemorrhage. Bleeding is caused by a combination of lysis of fibrin in wounds and of a systemic hemostatic defect that occurs as a consequence of plasmin-mediated proteolysis of plasma coagulation factors. The systemic hemostatic defect is characterized by hypofibrinogenemia, a reduction in the levels of factors V and VIII, an increase in fibrinogen-split products (which causes defective fibrin polymerization), and possibly by a platelet function defect.

In recent years, a new plasminogen activator has been developed that has potential advantages over streptokinase and urokinase because it can produce fibrinolysis without inducing a generalized hemostatic defect. This enzyme, known as extrinsic tissue plasminogen activator (t-PA), is a serine protease that has been isolated from a human melanoma cell line and has been produced for clinical uses by recombinant DNA technology. t-PA binds to fibrin with a greater affinity than either urokinase or streptokinase and when bound to fibrin, it is activated and converts plasminogen to plasmin on the fibrin surface.

Investigations in animals and in humans have demonstrated that the infusion of melanoma cell–derived t-PA is associated with thrombolysis without a significant generalized coagulation defect. These findings have recently been confirmed in studies of DNA-recombinant t-PA.

The potential advantages of use of t-PA rather than
urokinase and streptokinase as a thrombolytic agent is
predicated by its ability to induce fibrinolysis without
inducing a plasma proteolytic state. The importance of
this potential advantage is critically dependent on the
following relative contributions to bleeding associated
with therapeutic thrombolysis: (1) local fibrinolysis in
hemostatic plugs and (2) the plasmin-induced general-
ized coagulation defect. To date, the relative contribu-
tions of these mechanisms to hemorrhage caused by
thrombolytic therapy has not been defined. We have
performed a study in which the hemorrhagic effects of
equivalent thrombolytic concentrations of streptoki-
Ease and t-PA were compared in rabbits.

Materials and methods

Materials. t-PA was derived from the culture fluid of human
melanoma cells according to the method of Rijken and Collen. Its
specific activity was determined by assessing its dose-related
fibrinolytic effects on plasminogen-enriched bovine fibrin
plates and was expressed as international units as compared with
known amounts of urokinase. Streptokinase (Streptase; lot No.
0180A) was obtained from Behringwerke AG, Marburg,
F.D.R. The chromogenic substrate (82251, lot No. 85794 51)
and the human plasmin (25 caesin U/bottle; lot No. 6728951)
used in the α2-antiplasmin activity assays were obtained from
Kabi Diagnostica, Stockholm, Sweden. 125I (Na251 610
mCi/ml, carrier free) was obtained from New England Nuclear,
Boston. Sodium pentobarbital was obtained from MTC Phar-
maceuticals, Hamilton, Ontario. Fibrinogen (>90% clottable)
was prepared from pooled rabbit plasma and labeled with 125I by
the iodine/monochloride method. 20 All animal studies were
performed with New Zealand white rabbits and conformed to
the guiding principles of the American Physiological Society.

Method of preparation of radioactive jugular vein
thrombi. Standard-sized, preformed 125I-labeled thrombi were
produced in the external jugular veins of rabbits (2.3 to 3.5 kg).
The procedure used to produce radiolabeled thrombi was as
follows: Both external jugular veins were exposed through a
paramedial incision in the neck. Each vein was cleared over a
distance of 2 cm and small side branches were ligated. A 3.0-Ti-
cron braided polyester thread, presoaked in a collagen solution,
was then introduced lengthwise in the lumen of the jugular vein.
After 30 min the vein was clamped both proximally and distally
to isolate the vein segment. Then 0.15 ml of rabbit blood con-
taining 5 μl of 125I-labeled rabbit fibrinogen (approximately
500,000 cpm) was aspirated in a 1 ml syringe containing 1 U
of thrombin and 10 μl of CaCl2 (0.25M). This mixture was quickly
injected in the isolated jugular vein with the use of a 25-gauge
needle. In all instances the thrombus formed quickly and it was
allowed to age for 30 min before both vessel clamps were
removed. The restoration of blood flow was checked.

Characteristics of jugular vein thrombi

Urea solubility. Radioactive jugular vein thrombi were pre-
pared as described above. The thrombi were removed and the
solubility in 5M urea was tested. The thrombi were washed in
saline and then immersed in 5 ml 5M urea and the supernatant
was assayed for radioactivity at 0, 30, 60, 120, 240, and 360
min and 24 hr. Less than 1% of the radioactivity appeared in the
supernatant over the 24 hr incubation period.

Release of radioactivity during thrombolysis in vitro. To
determine whether the thrombi were uniformly labeled with 125I,
jugular vein thrombi were recovered and incubated in rabbit
platelet-poor plasma containing streptokinase in a final concen-
tration of 300 U/ml. The experiment was performed with eight
thrombi. Two thrombi were removed at 1, 2, 3, and 4 hr,
respectively, they were weighed, and the total radioactivity
measured. There was a parallel decrease in weight and total
radioactivity over the 4 hr period (r = .998).

Assessment of thrombolysis in vivo. Rabbits were infused
through the marginal ear vein with streptokinase (4000, 8000 or
16,000 U/kg/hr) or t-PA (7500 and 15,000 U/kg/hr) or an equi-
volume of suspending vehicle (saline). Ten percent of the total
dose of both fibrinolytic agents was administered as a bolus
loading dose and the remaining 90% of the total dose was
infused over the next 4 hr. (This dosage regimen was designed to
simulate regimens that have been successful in inducing
thrombolysis in patients with acute vein thrombosis and pulmo-
nary embolism. 1, 2) At the end of the infusion, the thrombi
remaining in the vessels were removed and their size was deter-
mined by measuring the amount of residual 125I-labeled fibrino-
gen remaining in the thrombi and by measuring their wet weight
and comparing the results with those of the saline controls.

Assessment of hemorrhagic side effects. Other rabbits were
injected with 1 ml of 52Cr-labeled red cells (2 × 109, 200,000
cpm). One hour later, the rabbits were anesthetized and given
either 4000 or 8000 U/kg/hr of streptokinase, 7500 or 15,000
U/kg/hr of t-PA, or an equivolume of saline. At the highest dose
of streptokinase (16,000 U/kg/hr), profuse bleeding occurred at
all wound sites, including ear puncture wounds, neck incisions,
and sites of venous cannulation. It was therefore technically
impossible to accurately quantitate the blood loss from the ear
puncture sites. As in the studies of thrombolysis, 10% of the
dose was administered as a bolus loading dose and 90% was
infused over the next 4 hr. Three minutes after the loading bolus
dose was administered and immediately before the continuous
infusion commenced, each rabbit’s ear was pierced five times
with a Bard-Parker No. 11 scalpel to produce five standard
9 mm full-thickness incisions. Each ear was then immersed in a
950 ml saline bath that was constantly stirred and maintained at
37°C. The amount of 51Cr-labeled red cells lost into the saline
bath from the puncture wound sites over the initial 15 min was
determined and expressed as initial blood loss. Further blood
loss into the saline bath was measured at 30 min intervals over
the next 4 hr. This method and reproducibility of its results have
been previously reported. 21

Assessment of systemic fibrinolysis. The effect of strep-
tokinase and t-PA on plasma proteolysis was determined by mea-
suring the level of α2-antiplasmin by the method of Teger-
Nielsen et al. 22 in which the chromogenic substrate S2251 was
used and by determining the thrombin clotting time (TCT). 23
Blood was withdrawn from the rabbit carotid artery directly into
3.8% sodium citrate (9/1, vol/vol). All samples were collected
before and 30, 60, 120, 180, and 240 min after the infusion
began.

Statistical methods. Data were analyzed by one- or two-way
analysis of variance or by the normal difference test, when
appropriate. The blood loss data were log-transformed to ac-
commodate increasing variation with increasing values. 24, 25

Results

The size of thrombus in saline-treated rabbits was 44
± 3 μl (volume, based on 125I radioactivity) and 34 ±
4 mg (wet weight) (mean ± SEM, n = 18). The size
of thrombus measured 4 hr later was unchanged (101
± 8% of initial size). For each set of experiments, the
size of thrombus was measured in the control animals
(saline infusion) and in the t-PA- and streptokinase-
treated animals. The size of thrombus in the animals treated with t-PA and streptokinase was expressed as a percent of the size of thrombus at 4 hr in saline-treated controls (i.e., the thrombus in saline controls at 4 hr was considered to be 100%). Streptokinase and t-PA produced a dose-related decrease in size of thrombus both when measured as volume based on radioactivity and as wet weight. Streptokinase, 4000, 8000, and 16,000 U/kg/hr, produced zero, 28 ± 6%, and 57 ± 4% thrombolysis, respectively, whereas 7500 and 15,000 U/kg/hr t-PA produced 35 ± 6% and 85 ± 4% thrombolysis, respectively (figure 1).

The effects of t-PA and streptokinase on initial and continuous blood loss are shown in figure 2. Streptokinase in doses of 4000 and 8000 U/kg/hr produced a significant increase in initial blood loss compared with that in saline-treated controls (p < .001). In addition, both lower streptokinase doses caused a progressive increase in bleeding that continued throughout the 4 hr of infusion (p < .0001). t-PA (7500 U/kg/hr) produced neither a significant increase in initial blood loss nor a progressive increase in continuous blood loss throughout the 4 hr of infusion. The larger dose of t-PA (15,000 U/kg/hr) had no effect on initial blood loss, but like streptokinase, produced a progressive increase in bleeding over the 4 hr of infusion (p < .0001). There were, however, a number of differences in the kinetics of bleeding in the animals treated with streptokinase and t-PA (15,000 U/kg/hr). Excessive bleeding was evident after both doses of streptokinase within 15 min of commencing the infusion, whereas bleeding was observed with t-PA only after 90 min of infusion. At 4 hr, blood loss with the two lower doses of streptokinase was 10,500 μl (range 600 to 14,200 μl) and 11,000 μl (range 400 to 23,000 μl); blood loss was 1400 μl (range 0 to 8100 μl) in the animals treated with 15,000 U/kg/hr of t-PA (p < .001; figure 2). With the lowest dose of t-PA (7500 U/kg/hr) blood loss was 144 μl (range 3 to 244 μl).

The effect of streptokinase and t-PA on α2-antiplasmin levels and the prolongation of the TCT are shown in figure 3. Streptokinase, 4000 and 8000 U/kg/hr, produced a significant decrease in α2-antiplasmin levels (p < .001) and a significant prolongation of the TCT (p < .001). t-PA, 7500 U/kg/hr, had no effect on either the α2-antiplasmin level or the TCT, but the 15,000 U/kg/hr dose produced a significant decrease in α2-antiplasmin level and a significant prolongation of the TCT. These effects were significant after 120 min of infusion (p < .01).

**Discussion**

Investigations in animals and in humans have demonstrated that the infusion of t-PA can produce thrombolysis without inducing a plasmin-mediated plasma proteolytic state.10-19 The results of our study confirm

**FIGURE 1.** Thrombolytic effects of 4 hr infusions of t-PA and streptokinase (SK). Thrombolysis was expressed as thrombus size (% of thrombus in saline controls), measured both in volume (based on amounts of 125I fibrinogen) and wet weight. Doses: t-PA7.5 = 7500 U/kg/hr; t-PA15 = 15,000 U/kg/hr; SK4 = 4000 U/kg/hr; SK8 = 8000 U/kg/hr; SK16 = 16,000 U/kg/hr. Data are expressed as mean ± SEM. Numbers in parentheses are n values.

**FIGURE 2.** Effect of saline, t-PA, and streptokinase on blood loss from punctured ear wound sites during the 4 hr of infusion. Data are expressed as geometric means due to increasing variation with increasing blood loss. Doses: t-PA7.5 = 7500 U/kg/hr; t-PA15 = 15,000 U/kg/hr; SK4 = 4000 U/kg/hr; SK8 = 8000 U/kg/hr. Numbers in parentheses are n values.
these observations since we demonstrated that t-PA, infused over 4 hr in a dose of 7500 U/kg/hr, produced thrombolysis without inducing a decrease in $$\alpha_2$$-antiplasmin level or a prolongation of the TCT. The thrombolytic effect of the lower dose of t-PA was not associated with an increase in blood loss when this was measured with a sensitive method that has been shown to be responsive to the hemorrhagic effect of heparin and thrombocytopenia. In contrast streptokinase produced an increase in blood loss at doses of 4000 and 8000 U/kg/hr, both of which induced less thrombolysis than t-PA in a dose of 7500 U/kg/hr. The higher dose of t-PA (15,000 U/kg/hr) given for 4 hr produced evidence of a plasmin-mediated proteolytic state, as demonstrated by a fall in $$\alpha_2$$-antiplasmin and a prolongation of the TCT. This finding is in keeping with predictions made by Sobel et al. that very high concentrations of t-PA are likely to induce a plasma proteolytic state. t-PA-induced proteolysis was associated with excessive bleeding. The pattern of this bleeding differed from that observed after streptokinase in that it was delayed for up to 90 min after the infusion began. The onset of bleeding coincided with a fall in $$\alpha_2$$-antiplasmin and a prolongation of TCT and therefore with the effects of a plasmin-mediated proteolytic state. However, blood loss in the rabbits treated with 15,000 U/kg/hr of t-PA was only 1400 $$\mu$$l compared with the mean 10,500 $$\mu$$l blood loss over 4 hr in the two groups of streptokinase-treated animals. Thus, when compared with streptokinase, t-PA had a greater thrombolytic effect but was less likely to produce hemorrhage according to blood loss measured both in the first 15 min and throughout the 4 hr of infusion.

The reason for the differences in bleeding observed with roughly equivalent thrombolytic effects of streptokinase and t-PA (8000 U/kg/hr and 7500 U/kg/hr, respectively) is not readily apparent. Excessive bleeding observed with streptokinase cannot be explained solely on the basis of a streptokinase-induced coagulation defect, since excessive bleeding was present 15 min after starting the infusion at a time when there was not a significant prolongation of TCT. It is possible, however, that the hyperplasminemic state induced by streptokinase produced bleeding by other mechanisms. Recently, Adelman et al. reported that streptokinase produced a plasmin-mediated defect in platelet membrane glycoproteins. It is possible, therefore, that the bleeding mediated by streptokinase is caused by a combination of lysis of fibrin in the hemostatic plug and a plasmin-induced platelet function defect. This proposed mechanism could also explain the bleeding observed with the higher dose of t-PA since it also produced a plasmin-mediated plasma proteolytic state. Alternatively, it is possible that fibrin present in the platelet-rich hemorrhagic plug is relatively resistant to lysis by t-PA because of high concentrations of platelet-derived fibrinolytic inhibitors. This hypothesis is supported by the results of recent studies that demonstrated that platelet thrombospondin combines with plasminogen and inhibits its activation.

The reason that bleeding was not induced by t-PA in a concentration that was able to produce approximately 35% lysis is less clear. It is possible that fibrin present in the hemostatic plug is more resistant to lysis by t-PA than fibrin in thrombi. Alternatively, the instability of the hemostatic plug during streptokinase therapy may be contributed to by a plasmin-mediated platelet function defect that is not produced in the animals treated with t-PA (7500 U/kg/hr). Whatever its mechanism, the absence of significant bleeding with thrombolytic...
concentrations of t-PA suggests that this plasminogen activator has considerable clinical potential.

References
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