A Long–Half-life and Fibrin-Specific Form of Tissue Plasminogen Activator in Rabbit Models of Embolic Stroke and Peripheral Bleeding

G. Roger Thomas, PhD; Harold Thibodeaux, BS; Carol J. Errett; Julie M. Badillo, BS; Bruce A. Keyt, PhD; Canio J. Refino, BS; Justin A. Zivin, MD, PhD; William F. Bennett, PhD

Background and Purpose We compared the activity of a new long–half–life, fibrin–specific tissue–type plasminogen activator (TPA) variant with that of wild–type TPA in rabbit models of embolic stroke and peripheral bleeding.

Methods In the embolic stroke model, TPA–induced clot lysis is followed by continuous monitoring of radiolabeled clot lodged in the middle cerebral artery. Twenty–four hours after embolization and treatment with either thrombolytic agent or excipient, the brains are removed, fixed, and evaluated for cerebral hemorrhage. In a parallel template bleeding time experiment, the effects of equipotent doses of the two TPA molecules were measured.

Results Infusion of wild–type TPA or bolus administration of the TPA variant resulted in dose–dependent clot lysis. The TPA variant was found to be an order of magnitude more potent than wild–type TPA on a milligram–per–kilogram basis. Unlike wild–type TPA, the variant caused less systemic activation of plasminogen (P<.05) and fewer hemorrhagic transformations in this model (P<.05). The TPA variant did not extend template bleeding times.

Conclusions These findings show that by combining increased fibrin specificity with decreased plasma clearance, it is possible to produce a thrombolytic agent that is more convenient and more potent than wild–type TPA. At the same time the significant reduction in hemorrhagic conversions may be attributable to the conservation of systemic plasminogen seen with this molecule. (Stroke. 1994;25:2072–2079.)

Key Words • cerebral hemorrhage • plasminogen • plasminogen activator, tissue-type • fibrinogen • rabbits

A n obstacle for universal clinical acceptance of thrombolytic agents for myocardial infarction is the risk of intracranial hemorrhage. This concern is greater when considering the use of thrombolytic agents in the treatment of embolic stroke where, even in untreated patients, hemorrhagic transformations occur in approximately one third of patients. Less serious, but possibly related to cerebral hemorrhage, is the problem of peripheral bleeding, both spontaneous and that which occurs from puncture wounds.

Bleeding, regardless of its physical location, is an occurrence to be avoided. Because of the limited number of patients who suffer severe intracranial hemorrhage in acute myocardial infarction, it has been difficult to define reliable clinical predictors. However, all the currently available thrombolytic drugs cause peripheral bleeding, and a dose–dependent increase in bleeding time is associated not only with clinical bleeding but also with activation of the systemic fibrinolytic system. We are attempting to design a thrombolytic drug that causes less bleeding, and it has been our experience that by increasing the fibrin specificity of tissue–type plasminogen activator (TPA), plasma fibrinogen levels can be preserved in vitro and, in a rabbit model, in vivo. The present study addresses the issue of whether bleeding can be decreased by increasing the fibrin specificity of TPA.

In addition to increasing the fibrin specificity, we also opted to use a slow clearing molecule that can be administered as a bolus. This is partly for convenience of administration and partly because extended infusions of wild–type TPA are one possible cause of clinical bleeding. Comparing the TIMI-II study, in which 150 mg TPA was used, with the GUSTO study using a 100–mg dose, it is apparent that the treatment regimens are very similar for the first 60 minutes; the major difference is that the last 60 mg in TIMI–II was given over an additional 5 hours. The difference in intracranial bleeding between these studies (2.10% versus 1.55%) suggests that extended infusions may be undesirable with respect to bleeding risk.

To address these problems, we describe some pharmacological properties of a new molecule designed to improve current thrombolytic therapy. The rationale behind the design of this thrombolytic agent was to develop a bolus–dose molecule with very high fibrin specificity to minimize systemic activation and thus reduce bleeding. The molecule, TNK, is based on the structure of human TPA with specific site–directed mutations, T103N, N117Q, and KHRR 296–299 AAAA. The alanine substitutions at 296–299 either alone or in combination with T103N have previously been shown to confer fibrin specificity. Addition–
ally, the TPA variant T103N, KHRR 296-299 AAAA has also been shown to be cleared 5.4-fold less rapidly than wild-type TPA in rabbits.14 Hotchkiss et al19 and others20 reported on the extended half-life of TPA that is achieved by substituting glutamine for the asparagine normally found at site 117 in the kringle-1 domain. By combining all of these modifications into one molecule, we have created a new thrombolytic agent that has a greatly reduced clearance (8-fold less than wild-type TPA) due to the addition of a complex carbohydrate attachment site at 103 and deletion of a high mannose glycosylation site at 117. Meanwhile, the properties of high fibrin specificity and resistance to plasminogen activator inhibitor-1 (PAI-1) conferred by alanine substitutions at 296-299131418 are conserved.

In this article, we compare TNK with wild-type TPA. We describe the kinetics of cerebral clot lysis and the frequency and magnitude of hemorrhagic transformations that occur after treatment of embolic strokes and comment on the propensity of TNK for causing peripheral bleeding.

Materials and Methods

Dihydrofolate reductase (DHFR)-deficient Chinese hamster ovary (CHO) cells, originally derived from CHO,21 were cotransfected with DHFR-expression plasmid and a plasmid containing the TNK expression cassette.27 After transfection, colonies were selected based on the expression level of TNK. The best clones were expanded for scale-up and production. The harvested cell culture fluid was diafiltered, and the TNK was purified using lysine affinity chromatography. Plasma clearance rates in rabbits, in vitro fibrin specificity, and PAI-1 resistance of TNK were verified as described by Keyt et al.19

Rabbit Embolic Stroke Model

The rabbit model for embolic stroke used in these experiments has been described in detail.23 Male New Zealand White rabbits (2.8 to 3.2 kg) were anesthetized with Hypnorm (0.7 mL/kg IM; Jansen Pharmaceuticals). The first lateral branch of the external carotid artery was cannulated retrogradely with Micro Renathane (Braintree Scientific Inc), and the tip was advanced to the carotid bifurcation. All accessible branches of the internal and common carotid arteries were ligated. Blood pressure was monitored continuously using a MacLab Data Recording System (AD Instruments Pty Ltd) through a catheter placed in the central ear artery.

Rabbit whole blood clots were formed in vitro by adding 0.5 mL of 51Cr-labeled sulfur colloids (2.5 mCi; Mallinckrodt Medical Inc), 0.25 mL calcium chloride (1 mol/L; Sigma Chemical Co), and 2 U bovine thrombin (Parke-Davis) to 1 mL whole blood drawn onto 0.1 mL of 3.8% sodium citrate (Mallinkrodt Inc). The clot was formed in polyethylene (PE 160) tubing (Clay Adams) and incubated for 1 hour at room temperature. The clots were extruded from the tubing and then cut into 5-mm sections, weighed, and washed by being rocked gently for 1 hour in 50 mL phosphate-buffered saline containing 1 mg/mL bovine serum albumin (Sigma Chemical Co). The radioactivity in the clots was then measured using a CRC-12 dosimeter (Capintec Inc). Clots were not used for embolization if they did not contain 5±1 μCi of radiolabeling material.

A single labeled clot per rabbit was introduced into the cannula and flushed into the cerebral circulation with 3 mL sterile saline (Elkins-Sinn Inc). Rabbits were then placed supine under anesthesia. Two minutes after injection, parallel-hole parallel-hole parallax images of the emboli were obtained with an Ohio Nuclear Pho-y camera (Siemens Medical Services Inc) using an energy window that encompassed the emission peaks of 51Cr. The position of the emboli within the middle cerebral artery was verified by comparing the coordinates of the embolus image with those of external markers on 5-minute static scintigrams. Once the placement was verified, a control 5-minute scintigram was collected, and then TPA, TNK, or excipient was administered. Throughout the experiment the data were collected and stored as a series of 5-minute scintigrams by using NAC software (Advanced Medical Computing). The kinetics of thrombolysis were calculated by analyzing the scintigrams after constructing a region of interest over the embolus. The disappearance of the radiation from the embolic site was normalized for 51Cr decay and calculated as a percentage of the radiation at time zero. Percentage of clot lysis was then calculated as 100% minus the percentage of radioactivity at time zero for each time point.

Dose-response curves for the activity of TPA and TNK were constructed. TPA (1.0 mg/mL; Activase alteplase, Genentech Inc) was administered as a loading bolus followed by an infusion. The initial bolus was equal to 10% of the hourly infusion rate. Infusion rates of 0.3 mg/kg per hour for 3 hours, 1 mg/kg per hour for 3 hours, and 3 mg/kg per hour for 2 hours were given without correction for volume. The maximal total volume was 6.3 mL/kg. These dosing regimens resulted in total doses of 0.93 (n=6), 3.1 (n=6), and 6.3 (n=6) mg/kg, respectively. TNK was given as a single bolus injection without correction for volume. The number of animals in each TNK treatment group represents the sum of all experiments conducted with protein from four small-scale research batches (B1 through B4). These batches had a protein concentration of 0.69±0.26 mg/mL (mean±SEM). The doses of TNK given and the number of rabbits receiving protein from each batch were as follows: 0.01 mg/kg, n=3 (5xB4); 0.03 mg/kg, n=9 (3xB2, 6xB4); 0.1 mg/kg, n=13 (7xB2, 6xB4); 0.3 mg/kg, n=12 (6xB1, 6xB4); and 0.6 mg/kg, n=34 (5xB2, 6xB3, 6xB4, 5xB3, 12xB4). The excipient (0.015% Tween 80 in 200 mmol/L arginine hydrochloride titrated to pH 7.5 with phosphoric acid) was identical for both TPA and TNK. Control animals (n=12) were given excipient as a 0.3-mL/kg bolus followed by a 3-mL/kg-per-hour infusion for 2 hours. Rabbits in each treatment group were assigned prospectively and without knowledge of ongoing hemorrhage rates within each group.

Intracerebral Hemorrhages

After treatment with TPA, TNK, or excipient and 2 hours of imaging as described above, the rabbits were allowed to recover for a period of 24 hours. They were then euthanatized, and the brains were removed. The brains were fixed in 10% formalin for a minimum of 2 weeks, checked for gross surface hemorrhages, and cut into 2.5-mm sections. The sections were observed for hemorrhage and scored by personnel blinded to the treatment groups according to the presence of a hemorrhage on one side or both sides of each section. A hemorrhage apparent on one side was given a score of 1. If the hemorrhage was continuous to the other side of the section, then a score of 2 was given. The total score included all the section scores and was thus assigned to each brain. This technique identifies visible cerebral hemorrhage but does not differentiate between intracerebral hematomas and hemorrhagic infarction.

Peripheral Bleeding

The effects of excipient (0.3-mL/kg bolus plus 3 mL/kg per hour for 2 hours), TPA (0.3-mg/kg bolus plus 3 mg/kg per hour for 2 hours), or TNK (0.6 mg/kg) on template bleeding times were measured in the shaved ears of a separate group of male New Zealand White rabbits. The experiments were conducted in conscious restrained animals. Measurements were made at 1, 5, 30, 60, 90, and 120 minutes after the start of the administration of the agent. Template bleeding times were measured using Surgicutt blades (International Technidyne Corp) to make a 5-mm-long cut 1 mm deep in the ear. The incisions were wicked every 15 seconds with Surgicutt blotting paper (International Technidyne Corp). Only the
Fig 1. Graphs show dose-dependent effects of tissue plasminogen activator (t-PA, TPA) and TNK for cerebral clot lysis as measured by the disappearance of 99mTc radioactivity in a rabbit embolic stroke model. TPA and excipient were administered as a loading bolus followed by an infusion (over either 2 or 3 hours) (A), while TNK was given as a single bolus (B). The data are presented as mean±SEM of 6 animals for TPA, 12 excipient controls, and from 3 (0.01 mg/kg) to 34 (0.6 mg/kg) animals for TNK. In panel B, the data for 0.3 mg/kg overlap those for 0.1 and 0.6 mg/kg and have been omitted for the sake of clarity.

middle portion of the dorsal surface of the ear was used. The occurrence of rebleeding from the wounds was also noted. All animal studies were approved by the institutional animal care and use committee of Genentech Inc.

Plasminogen, Fibrinogen, α2-Antiplasmin, and TPA Determinations

In animals chosen at random from the stroke study, blood samples were taken at 1, 5, 30, 60, 90, and 120 minutes after the initiation of treatment. These samples were used for the measurement of TPA antigen levels and to monitor the effects of TPA (n=4) and TNK (n=7) on plasma plasminogen, fibrinogen, and α2-antiplasmin (α2-AP) as described previously.23

Statistical Analysis

A two-tailed Student’s t test was used to analyze differences in time to 50% clot lysis, and a Fisher’s exact test was used to determine the significance of the reduction in the frequency of hemorrhagic transformation. Changes in template bleeding time and systemic activation parameters were assessed by two-way ANOVA for repeated measures followed by a protected least significant difference test. In all cases a probability value of less than .05 was considered significant.

Results

Fig 1 indicates that TPA and TNK exhibit dose-dependent clot lysis properties. Additionally, it can be seen from the kinetics of clot lysis that TPA (3 mg/kg per hour for 2 hours [6.3 mg/kg]) and TNK (0.6 mg/kg) give almost complete clot lysis as measured by the disappearance of radioactivity and that this occurs over equivalent time intervals. In each case the time taken for 50% lysis of the embolus is 35±5 minutes for TPA, and 48±6 minutes for TNK (P>.05). These doses thus have similar thrombolytic activity, suggesting that TNK is at least an order of magnitude more potent than TPA.

Because it is difficult to directly compare the results of drugs administered by bolus versus infusion, the potency of TNK with respect to TPA can be better estimated by calculating the amount of clot lysis that occurs after a given duration of treatment for each animal. In this case a treatment period of 60 minutes was chosen because it is on the linear portion of the lysis curves (Fig 1). These values were averaged for each treatment group and plotted against the amount of drug received in the first hour, as shown in Fig 2. The amount of drug needed to achieve 50% lysis in 60 minutes was 1.3 and 0.06 mg/kg for TPA and TNK, respectively. Therefore, on a milligram-per-kilogram basis, TNK appears to be approximately 20 times more potent than TPA in this occlusive embolus model. From data on plasma antigen level (Fig 3), clearance values of 2 and 15 mL/min/kg can be calculated for TNK and TPA, respectively. These values are comparable to earlier published estimations of 1.9 and 16.1 mL/min/kg.17 When 1.26 and 0.064 mg/kg are divided by clearance to give area under the curve (AUC, an appropriate measure of the animal’s exposure to the drug) then TNK
(0.032 mg • min/mL) is approximately equipotent with TPA (0.084 mg • min/mL).

Subsequent comparisons of possible bleeding-related side effects between the two molecules were made using a supramaximal dose of TNK (0.6 mg/kg) and a maximally effective dose of TPA (0.3-mg/kg bolus plus 3 mg/kg per hour over 2 hours, 6.3 mg/kg total) (Fig 2). In 6 rabbits given excipient, 3 showed intracranial bleeding. Although treatment with TPA (6.3 mg/kg) effected timely lysis of the cerebral embolus, it did not significantly affect either the frequency of hemorrhagic transformation (33%) or the size of the resultant hemorrhage (7.0 faces for excipient versus 4.5 faces for TPA) (P >.05). However, TNK (0.6 mg/kg) caused significantly fewer hemorrhages compared with those in excipient-treated animals (P <.05). In the TNK group, evidence of bleeding was seen in only 13% of brains with an average size of 10 faces when examined on necropsy 24 hours after treatment. In all cases where hemorrhagic transformation occurred, the cerebral hemorrhage was of the hemorrhagic infarction type. No intracerebral hematomas were observed in any treatment group.

The differences in hemorrhagic conversion rates are unlikely to reflect differing hemodynamic profiles of the two drugs. However, it should be noted that TPA (6.3 mg/kg) and TNK (0.6 mg/kg) caused small but consistent depressor responses of 7.3 ±1.1 and 9.7±1.9 mm Hg from baseline mean arterial pressures of 78±2.0 and 76±1.6 mm Hg, respectively. Excipient infusions did not cause changes in mean arterial pressure.

Analysis of the kinetics of the clot lysis showed that there was some variability between the four preparations of TNK used in this study. One indication of this variability was that, within the 0.6-mg/kg dose group, the time to achieve 50% clot lysis ranged from 18 to 120 (maximal) minutes. In every case, hemorrhage was associated with individual rabbits that had extended times to 50% clot lysis (75, 78, 98, and 100 minutes). This occurred when the template bleeding time measurement from a baseline value of 1.5±0.2 minutes (P >.05) (Fig 5) (P >.05). The actual ischemic period is given by time to 50% clot lysis plus 20 minutes. This increase, although significant, is short-lived and hemostasis returns to normal at 60 minutes (2.7±0.4 minutes) (Fig 5). In animals treated with TPA, the wounds, although having stopped bleeding long enough to establish a bleeding time, did rebleed throughout the experiment. In contrast, an equipotent dose of TNK (0.6 mg/kg) given as a bolus did not cause any significant augmentation of this bleeding time measurement from a baseline value of 1.5±0.2 minutes (P>.05) (Fig 5). It was also observed that once the wounds had stopped bleeding in the TNK group, there was no further rebleeding.

In plasma samples taken from rabbits treated with either TNK (0.6 mg/kg) or TPA (6.3 mg/kg), it can be seen that TPA caused a significant degree of systemic activation compared with TNK. At 2 hours, the circulating levels of plasminogen, fibrinogen, and α2-AP had decreased to 43±4%, 55±5%, and 37±6% of initial levels, respectively, in wild-type TPA-treated animals. On the other hand, the fibrin-specific variant 
Discussion

It has been proposed that fibrin specificity, PAI-1 resistance, and reduced clearance are desirable qualities in a new thrombolytic agent. One approach to producing a molecule with some of these qualities is the characterization of vampire bat plasminogen activator (rDSPA). This activator exhibits increased fibrin specificity; however, the clearance rate of rDSPA is rapid. Furthermore, it is a foreign protein with some risk of immunogenicity. In contrast, we used protein engineering techniques to modify the endogenous human protein to produce TNK.

In our models, TNK has minimal systemic effects. This is a similar result to that seen in rats for rDSPA and confirms that the fibrin specificity of other KHRR 296-299 AAAA-containing variants is conserved in this molecule. At therapeutic doses, the conservation of systemic plasminogen, fibrinogen, and α2-AP is coincident with a reduction in hemorrhagic transformations in an embolic stroke model as described for KHRR 296-299 AAAA and no increase in template bleeding times.

The reason for using this model was twofold. First, in a human population of myocardial infarction patients treated with a therapeutic dose of TPA, there is an approximately 1% to 2% chance of developing a stroke. Thus, we used a model for studying the potential of new thrombolytic agents to alter hemorrhagic risk in a system where both beneficial and detrimental effects can be seen in a small group of animals. We found that in our model, based on the earlier work of Zivin and coworkers, embolic stroke induced cerebral bleeds in 50% of the control (excipient-treated) animals. The mechanism by which these hemorrhages occur is unknown. However, wild-type TPA does not change this frequency significantly. Second, there is increasing interest in the use of TPA for thromboembolic stroke. Similar models have been used extensively in other laboratories, with great success, to study the efficacy of TPA for cerebral thromboembolic lysis. However, in studies carried out thus far, there have been no reports of any commercially available thrombolytic agent that reduces significantly the rate of hemorrhagic transformations. We have reported previously that a fibrin-specific TPA variant, KHRR 296-299 AAAA, does not cause hemorrhagic transformations when used in this stroke model. However, this is the first report using a bolus administration of a slow-clearing, more fibrin-specific plasminogen activator to reduce hemorrhagic transformation in a model of thromboembolic stroke.

TNK has a clearance rate 8-fold less than that of TPA. The advantage of bolus administration of a more fibrin-specific thrombolytic agent is that higher plasma concentrations can be attained without increasing the risk of inducing systemic plasminogen activation. The higher plasma concentrations are also more likely to produce more rapid clot lysis. To attain high plasma concentrations using TPA, front-loaded accelerated infusion regimens have been used clinically for myocardial infarction. This results in high coronary patency rates but at the cost of significant reduction in the circulating components of the fibrinolytic system, especially α2-AP.

It was seen that bleeding times were elevated during the first hour of TPA infusions with peak prolongation occurring at 5 minutes. The reason why TPA causes this response is not entirely clear but may be due to the rate of production of fibrinogen degradation products. The increase in circulating levels of fibrinogen degradation products may be enough to cause a direct effect on platelets by competing with fibrinogen at the GP IIb/IIIa receptor during clot formation. Reactivated PAI-1 inhibits the extension in bleeding times caused by the coadministration of TPA and aspirin. Additionally, we and others have found that aprotinin (an inhibitor of plasmin but not TPA) reverses the TPA-induced increase in bleeding. This supports the theory that circulating plasmin is involved in the increase in bleeding times. All of this suggests that bleeding times are not elevated after TNK because of the increased fibrin specificity of the molecule and its decreased rate of activating systemic plasminogen.

This ability to limit the levels of circulating plasmin could also account for the reduction in the incidence of hemorrhagic transformations.
hemorrhagic transformations seen in this study. However, there is mounting evidence that the cerebral endothelial cells respond differently to the presence of TPA compared with their peripheral counterparts. deBono et al. showed that TPA-induced cerebral bleeding as a result of injury was inhibited with tranexamic acid, whereas aprotinin was more potent in reducing peripheral bleeding times. Further evidence was presented by Chehrazi et al., who showed TPA to be more potent at lysing equivalent clots in the central nervous system compared with clots in the peripheral vasculature. Thus, the reason for reduced cerebral hemorrhages may be more complicated than simply attenuating circulating plasmin levels.

Close examination of the pattern of cerebral bleeding seen in our study when using TNK and TPA suggests that more than one mechanism probably is involved. From these data it appears that if cerebral clots can be lysed early—in this case, 50% lysis within 90 minutes (20 minutes between embolization and treatment and 70 minutes after giving TNK)—by using a fibrin-specific plasminogen activator, then there is a very low risk (2%) of hemorrhagic transformation. However, that risk is increased to 50% if the time to 50% lysis is extended. The same time-dependent risk of hemorrhage is not seen in wild-type TPA-treated animals, suggesting that even when emboli are rapidly dissolved, unless there is extreme fibrin selectivity, the risk of hemorrhage is high. It is currently uncertain how these data will translate to human studies, but early indications show that patients treated within 90 minutes with TPA may show early neurological improvement. Although the risk of hemorrhagic transformation remains significant, this too could be reduced in the future by the use of fibrin-specific plasminogen activators such as TNK. Another factor that can significantly affect the outcome of stroke patients receiving thrombolytic agents is the use of adjunct therapies such as aspirin or heparin. It has been shown previously that aspirin, but not heparin, can reduce the risk of cerebral hemorrhage in this model when TPA is used to lyse cerebral emboli. However, it is not known how these agents will interact with highly fibrin-specific thrombolytic agents such as TNK.

In conclusion, we have shown that a fibrin-specific plasminogen activator molecule, engineered to be cleared slowly, when given as a single bolus works as efficiently as wild-type TPA given under optimal conditions of a 2-hour infusion. Furthermore, we find that by increasing the fibrin specificity of TPA we are able to reduce its propensity for causing peripheral bleeding and ameliorate the risk of cerebral hemorrhages in a rabbit model of embolic stroke. These findings are encouraging first steps toward developing a new potent, convenient, and safe thrombolytic therapy.

Acknowledgments

The authors wish to thank Dr John Ogez and Dr Tina Etcheverry for their work on expression, production, and purification of the TPA variant. In addition, we extend our gratitude to Dr David Giltinan for his assistance with the statistical analysis. We are also indebted to Luis Peña, Alice Chow, Jadine Lai, Lea Berleau, Hung Nguyen, and Cheryl Pater, who all made significant contributions to the development of the TNK variant of TPA.

References


Editorial Comment

At this writing, several randomized, placebo-controlled trials of intravenous thrombolytic therapy in patients with acute ischemic stroke being conducted worldwide are nearing completion. The results of these studies are anxiously awaited, since evidence of efficacy would represent a major advance in the management of stroke, even if applicable to only a small percentage of patients.

If efficacy is ultimately demonstrated, a major stumbling block to the wide dissemination of this treatment may be the associated rate of adverse bleeding complications, particularly intracerebral hemorrhage. In open-label trials of intravenous recombinant, wild-type, tissue-type plasminogen activator (tPA) administered to patients with very early ischemic stroke (<3 hours), the rate of symptomatic hemorrhagic change approached 5%. While there undoubtedly is a low (but uncertain) rate of symptomatic hemorrhagic change in patients managed in a standard fashion without thrombolytic therapy that would tend to abrogate some of this risk, it seems likely that symptomatic intracranial hemorrhage will complicate thrombolytic treatment of acute ischemic stroke more frequently than it does the treatment of myocardial infarction. In the latter case, it has been proposed that fear of complications is one factor that has resulted in the withholding of this proved beneficial treatment for myocardial infarction from nearly 100 000 Americans annually.3

In this regard, the accompanying article from Thomas and colleagues comes as encouraging news. Bolus intravenous administration of a more potent, more fibrin-specific, and longer half-life plasminogen activator (termed TNK) in a rabbit model of embolic stroke was associated with a lower risk of gross pathological intracerebral hemorrhagic change (13%) than in rabbits similarly treated with either excipient (50%) or continuous infusions of wild-type tPA (33%). While the difference in hemorrhage rates between TNK- and tPA-treated animals was presumably not statistically significant, the difference between TNK and excipient was P<0.05. The difference between tPA and excipient was not statistically significant. Moreover, TNK administration did not appear to prolong template bleeding times of the animals.
A long-half-life and fibrin-specific form of tissue plasminogen activator in rabbit models of embolic stroke and peripheral bleeding.
G R Thomas, H Thibodeaux, C J Errett, J M Badillo, B A Keyt, C J Refino, J A Zivin and W F Bennett

Stroke. 1994;25:2072-2078
doi: 10.1161/01.STR.25.10.2072

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/25/10/2072

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke 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 Stroke is online at:
http://stroke.ahajournals.org//subscriptions/