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Biochemical Dynamics Relevant to the Safety of Low-Dose, Intraclot Alteplase for Deep Vein Thrombosis

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Abstract

Intraclot tPA has been shown to be an effective treatment for DVT (Radiology 2008;246:619 & J Vasc Interv Radiol 2011;22:1107). We sought to correlate pharmacokinetics of tPA, fibrinogen, fibrinolytic inhibitors, and D-dimers with the safety and efficacy of intra-clot tPA. Thirty subjects received intraclot tPA for lower extremity DVT by infiltrating the thrombus with 10 mg doses tPA in an open-label study, using a pulse-spray catheter. We measured various parameters over 8 hours following a first dose of tPA. Mean tPA levels of 75 units per mL (95% confidence interval 19–131 units/mL) were seen immediately after administration of a mean tPA dose of 8.0 mg (SD 1.5 mg). tPA levels returned to baseline within 2 hours of completion of treatment. PAI-1 was consumed following tPA treatment, but rose to levels significantly greater than baseline ($P < 0.001$). Fibrinogen decreased slightly, but remained > 125 mg/dL for all subjects. α_2 -antiplasmin decreased from a mean of 115 units/mL to 56 units/mL after tPA administration ($P < 0.001$), and remained decreased for 8 hours. Plasminogen at baseline (112 units/mL) decreased to 89 units/mL immediately after tPA administration ($P < 0.001$), and was unchanged thereafter. D-dimer levels were > 20 μ g/mL in all but four subjects, one of whom was the only one to fail to achieve clot lysis. The safety of low-dose, intra-clot tPA is due to its short persistence in the circulation, lack of hypofibrinogenemia, and a reflexive rise PAI-1. Subjects whose D-dimers remain < 20 μ g/mL are at risk of not achieving thrombolysis.

Introduction

The goal of thrombolytic therapy for deep vein thrombosis (DVT) is to reduce the incidence of irreversible vein damage that often results when anticoagulation is given alone (1, 2). Reluctance to use thrombolytic agents has arisen from the fear of major bleeding, which is estimated to occur in 8% of cases (1). Hemorrhage results when thrombolytic enzymes remain in the general circulation long enough to lyse fibrin plugs necessary for physiologic hemostasis. Nevertheless, thrombolytic therapy for iliofemoral DVT is gradually gaining

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acceptance (1). A nation-wide clinical trial is underway to test the efficacy of enzymatic thrombolysis combined with mechanical thrombectomy (3). Encouraging results from other trials have recently been published (4–8).

To reduce bleeding risk alteplase (Activase; Genentech, South San Francisco, CA) is now often chosen as the thrombolytic agent because alteplase (in contrast to urokinase and streptokinase) localizes to thrombi by selectively binding to fibrin in preference to fibrinogen. To maximize this binding, and to assure that it reaches clot that is not in contact with circulating blood, alteplase is usually given by direct injection into thrombi through transvenous catheters rather than by peripheral intravenous infusion. Any drug that escapes into the general circulation is expected to disappear with a half-life of ~ 5 minutes (9).

Since Agnelli, et al., observed in a rabbit model that continuous infusion of alteplase is not necessary for continuous thrombolysis (10, 11), we developed thrombolytic regimens in which small boluses of alteplase are deposited throughout the entire thrombus by multiple “pulse-spray” injections over 1 – 4 hours depending on the extent of the thrombi. To date we have published the results of two trials in which our regimens were both highly effective and safe (7, 8). During the more recent trial we collected blood samples for several hours after the first administration of alteplase in part to monitor the appearance and clearance of tPA and to see if it returned to the circulation during clot lysis (12). We also measured the effect of tPA on various fibrinolytic plasma proteins to enhance our understanding of the dynamics of intraclot delivery of tPA. In this paper we report the results from the 30 patients in our second (“low-dose”) tPA trial who were treated with up to 10 mg of alteplase per dose (8). All but one patient had successful clot lysis, and none of them had serious bleeding complications (Table 1).

We believe that this is the only description of the molecular dynamics of intraclot alteplase treatment for DVT, and the results may be helpful in design of future trials of thrombolytic therapy and explain, in part, why the method is apparently so safe.

Materials and Methods

Human subjects

Subjects were enrolled in an open-label, non-randomized, phase II protocol for the treatment of lower-extremity deep vein thrombosis under NIH Clinical Center protocol 04-CC-0178 (registration NCT00082355 at www.clinicaltrials.gov). A detailed description of the protocol including the inclusion and exclusion criteria and clinical results has been published previously (8). The protocol was approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute, Bethesda, MD. All participants gave written informed consent to participate. An IND exemption for the use of alteplase in this research study was held sequentially by M. Horne and R. Chang.

Study design

10 mg of tPA was diluted to a concentration of 0.1 mg/ml in normal saline (100 ml total volume), then injected into the clot via a transvenous pulse-spray catheter (Angiodynamics Corp., Queensbury, N.Y.) in 0.5–1.0 ml aliquots until the entire clot was infiltrated. An attempt was made to infiltrate the entire clot with tPA during each treatment session to maximize clot lysis and restore flow as quickly as possible. Clots that were symptomatic for less than 7 days typically lysed completely with an initial injection and one follow-up treatment. Clots that were symptomatic for longer periods of time (up to 14 days) typically were injected 2 or 3 times. tPA administration after the first treatment was guided by venography to visualize the remaining clot; segments that were resistant to initial lysis were

injected 2 or 3 times on subsequent treatment sessions, up to a total tPA dose of 10 mg for each day of treatment.

Immediately before alteplase administration began, immediately after treatment completion, and 1, 2, 4, and 8 hours thereafter, venous blood was drawn through an internal jugular catheter flushed only with saline, and used solely for sample collections.

Laboratory assays

Blood was collected into Biopool Stabilyte vacuum tubes (3.2% sodium citrate, pH 6, Trinity Biotech, Jamestown, N.Y.) for measurement of tPA and PAI-1 activities using Chromolize tPA and PAI-1 kits from Biopool (Umea, Sweden). Samples for measurements of fibrinogen, plasminogen, and α 2-antiplasmin activities and D-dimer antigen were collected into BD Vacutainer Blood Collection Tubes (4.5% sodium citrate, Becton Dickinson, Franklin Lakes, NJ). Initially samples for fibrinogen were collected into citrate with epsilon aminocaproic acid (EACA) to prevent possible *in vitro* fibrinogenolysis. However, in a study (not shown) we found that fibrinogen activities in plasma collected with and without the fibrinolytic inhibitor were not significantly different. Further, there was no decrease in fibrinogen levels over two hours at ambient temperature in samples spiked with tPA after collection in sodium citrate alone. Fibrinogen measurements were therefore made in plasma collected in sodium citrate alone. Plasma samples were frozen in aliquots at -70°C until the assays were performed. The protein activities were measured with a STA-R Evolution analyzer manufactured by Diagnostica Stago (Parsippany, NJ), and the D-dimer antigen was measured using the STA-Liatest D-Di (Diagnostica Stago).

Statistical Analysis

The statistical significance of protein changes occurring during the course of alteplase administration were determined by t-tests.

Results

Systemic tPA and PAI-1 activity levels

Immediately after completing their first intra-clot injection of up to 10 mg of alteplase the mean pre-treatment tPA activity in the 30 patients had risen to 75 units/mL (SD = 56 IU/mL) from a mean pre-treatment level of 0.9 units/mL (Fig. 1). The first dose of tPA for each patient was based on the amount of venous thrombosis evident on the initial venograms, and limited to a maximum dose of 10 mg, per protocol. The mean first tPA dose was 8.0 mg (SD = 1.5 mg), ranging from 5 mg to 10 mg. Only 8 of the 30 patients required the maximum permitted daily dose of 10 mg. In all cases tPA activity declined to near-baseline levels by 1 hour after treatment and was at baseline within 2 hours after the dose (Figure 1).

Pre- and post-treatment PAI-1 activities in plasma collected at the same time points over an 8 hour period after alteplase administration are displayed in Fig. 2. Mean pre-treatment PAI-1 levels of 7.4 units/mL fell to essentially undetectable levels immediately after completion of alteplase administration. As tPA activity decreased over the following 1 – 2 hours (Fig. 1), mean PAI-1 levels rose rapidly (Figure 2) and by the 8 hour time point were three times the baseline values (26 units/mL, SD 17 units/mL, $P < 0.001$).

Fibrinogen

Pre- and post-treatment plasma fibrinogen levels are shown in Fig. 3. Immediately after treatment ($t = 0$) the mean fibrinogen level had decreased from 410 mg/dL to 350 mg/dL ($p < 0.01$). Thereafter fibrinogen levels remained essentially constant for 8 hours. The lowest fibrinogen measured in any patient was 125 mg/dL. To assess the effect of tPA on

fibrinogen levels measured in sodium citrate anticoagulated plasma (without any inhibitor of fibrinolysis), we spiked normal citrate anticoagulated blood with tPA in vitro, and assayed fibrinogen over two hours at ambient temperature to see if there was significant degradation of fibrinogen during transit to the laboratory. There was no decrease in fibrinogen (data not shown), indicating that in vitro degradation of fibrinogen did not cause the decrease in fibrinogen levels that we observed in our patients.

Plasminogen and α 2-antiplasmin activities

Although plasminogen activity dropped significantly after alteplase treatment (112 units/mL baseline vs. 89 units/mL after treatment, $P < 0.001$), it was never more than marginally below the reference range for the laboratory (67 – 127 IU/mL) at the completion of treatment and remained stable for the following 8 hours (Fig. 4). The decline, though statistically significant, was not clinically important. α 2-antiplasmin activity (Fig. 5) fell significantly ($P < 0.001$) during the course of alteplase administration. In contrast to plasminogen, the majority of patients had α 2-antiplasmin levels below normal (reference range, 75–132 IU/mL). Unlike PAI-1, there was no rebound of α 2-antiplasmin after tPA treatment and the values remained abnormally low, for the 8 hours following tPA administration.

D-dimer

In most of the patients D-dimer antigen levels rose abruptly by the time alteplase administration had been completed (Fig. 6). Only 4 patients failed to develop a D-dimer level of at least 20 μ g/mL. One of these four was the single patient who failed to achieve clot lysis.

Discussion

An hour after alteplase administration concluded, tPA activity in the general circulation of our patients was virtually absent. Nevertheless D-dimer concentrations continued to rise (Fig. 6), and venograms the next day revealed that much of the clot had disappeared in nearly all patients, proving that thrombolysis had continued without circulating alteplase (8). This is the outcome predicted by the rabbit model of Agnelli, et al., and demonstrates that continuous infusion of rtPA is not required for effective clot lysis in humans (10).

Although some active alteplase entered the circulation (Fig. 1), this disappeared quickly once drug delivery was complete, and even during the interval when alteplase was circulating, there was minimal evidence of tPA activity outside of the thrombosed veins. Moreover no significant bleeding developed in any of the patients. Plasma plasminogen fell very little (Fig. 4), for example, suggesting that most of the plasmin generated by alteplase was derived from plasminogen that had been incorporated into the thrombi as they formed (14). At the very most, fibrinogen (Fig. 3) fell slightly during the course of alteplase administration (from a mean baseline value of 410 mg/dL to a post-treatment value of 350 mg/dL), and then remained remarkably stable. This observation indicates that any plasmin leaving the thrombi after treatment was immediately inhibited by α 2-antiplasmin. Complexing of plasmin and α 2-antiplasmin probably accounts for the 50% reduction in plasma levels of the inhibitor (Fig 4, 5).

A surprising observation was the rapid rebound of PAI-1 activity (Fig 2). At the end of the treatment period (time 0) PAI-1 activity was very low, as expected if some alteplase had escaped from clots as the drug was administered. But within an hour PAI-1 had rebounded, and it remained elevated for at least the next 8 hours. The origin of this PAI-1 is unknown. It could have been secreted from platelets or from endothelium stimulated by the treatment

catheters or by a stimulant released from the lysing thrombi. PAI-1 is known to be an acute phase reactant, and its synthesis by the liver may have been increased in response to tPA. Maybe the liver responded with an acute phase reaction (15). Regardless of its source, excess PAI-1 was available to inhibit any alteplase that diffused into the circulation as the clots lysed. The naturally rapid clearance of rtPA ($T_{1/2} \sim 5$ minutes) was therefore not the only protection from systemic plasmin generation.

We believe that the rise in PAI-1 activity is an important factor in preventing systemic effects (e.g., bleeding) from intraclot injections of alteplase. In the group of patients we studied here we did not measure inhibited tPA (i.e., tPA:PAI-1 complexes). Therefore, we do not know whether alteplase continued to enter the circulation for a matter of hours as the thrombi lysed. However, in 4 patients treated with higher doses of alteplase (13) we found that tPA antigen (not activity) persisted for at least 12 hours after alteplase administration. Because tPA:PAI-1 complexes have a circulation half-life not much longer than that of free tPA, the persistence of tPA antigen for many hours indicated that tPA continued to reach the circulation for this length of time (16). If that was also true for the patients given 10 mg of alteplase, then during the post-treatment period PAI-1 activity was being continuously consumed as it inhibited newly arriving tPA and therefore must have been continuously generated.

It is important to point out that these data were collected after single doses of relatively small amounts of alteplase and should not be extrapolated to other treatment regimens. We cannot assume that the rebound of PAI-1 would recur if the treatments were given more frequently or continue if alteplase were given by continuous infusions into thrombi. Since fibrin degradation products (like fibrin itself) are reported to activate circulating tPA, the need for high levels of PAI-1 may be particularly important as thrombi continue to lyse and release D-dimer and tPA (12). It is possible that the source of PAI-1 would become depleted and need time to regenerate. Therefore, we advise that studies of molecular dynamics be included in the design of future trials of alteplase for DVT in order to enhance understanding of the clinical outcomes.

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All authors have read the journal's policy on conflicts of interest and have none to declare. We would like to thank Alison Cooper, NIH Clinical Center Department of Laboratory Medicine, for processing numerous samples for this study.

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Figure 1.
Plasma tissue plasminogen activator (tPA) activity before and after intra-clot administration of up to 10 mg alteplase (mean dose 8.0 mg) to 30 patients.

Figure 2.
Plasma plasminogen activator inhibitor-1 (PAI-1) activity in the same patients as in Fig. 1.

Figure 3. Fibrinogen levels in the same patients as Fig. 1. Normal reference range 177 – 466 mg/dL.

Figure 4.

Plasma plasminogen activity in the same patients as in Fig. 1. Normal reference range for plasminogen 67 – 127 IU/mL.

Figure 5.

Alpha-2-antiplasmin activity in the same patients as in Fig. 1. Normal reference range for alpha-2-antiplasmin 75 – 132 IU/mL.

Figure 6.

D-dimer concentrations in the same patients as in Fig. 1. Normal reference range $< 0.5 \mu\text{g} / \text{mL}$. Subject 2, marked by asterisk, failed to achieve venous patency with tPA treatment.

Table 1**Subjects, Therapy, and Outcomes of Low-Dose, Intra-Clot tPA**

Mean Age:	46 years (range: 18–62 years)
Gender:	18 males, 12 females
Duration of Symptoms:	10.8 days (range 7–16 days)
Success Rate (Patency):	29 of 30 (97%) had restoration of venous blood flow upon discharge after initial treatment with tPA.
Bleeding:	
Major	0/30
Minor	3/30 (10%) had hematomas at catheter insertion site. One subject had decreased tissue elasticity at site of catheterization due to prior radiation therapy for lymphoma. None required transfusion or interruption of tPA or anticoagulation. All resolved spontaneously.
Recurrence:	0/30 during six month study period.
Treatment Parameters:	
Mean Days of Treatment	2.6 days
Mean Daily tPA Dose	7.3 mg
Mean Total tPA Dose	19 mg