The Usefulness of Systemic Administration of Recombinant Human Tissue-Type Plasminogen Activator in Femoral Arterial Thrombolysis of Rabbits

Terumasa MINO, Junko SUZUKI and Haruki HAYASHI
Pharmaceutical Research Center, Toyobo Co., Ltd., Katata 2-1-1, Ohtsu, Shiga 520-02, Japan
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Abstract—The thrombolytic effect of locally or systemically administered recombinant human tissue-type plasminogen activator (rt-PA) was investigated in comparison with the effect of tissue culture urokinase (TCUK), using a model of femoral artery thrombosis in rabbits. An 125I-labeled fibrinogen thrombus was formed in the femoral artery following injury of the intima by diluted sulfuric acid, and thrombolytic activity was evaluated one hour after the end of infusion of the agents. Local infusion of rt-PA (500–10,000 IU/kg) and TCUK (10,000 IU/kg) induced a marked thrombolysis. When rt-PA and TCUK were injected systemically in a high dose (200,000 IU/kg), rt-PA but not TCUK had a significant thrombolytic activity. In these cases, rt-PA was not accompanied by systemic activation of the fibrinolytic system, as evaluated by unaltered levels of α2-antiplasmin in the plasma, while TCUK led to a substantial decrease in the α2-antiplasmin level. These results suggest that systemically administered rt-PA but not TCUK induce a significant thrombolysis without systemic activation of the fibrinolytic system in cases of peripheral artery thrombosis.

Recombinant human tissue-type plasminogen activator (rt-PA) produced by DNA technology has characteristics similar to those of human native t-PA.

It has been reported that t-PA had a relatively high fibrin-specificity in comparison with streptokinase or urokinase and that it showed potential thrombolytic activity without systemic fibrinogenolysis in experimental animals (1–5). Therefore, in preliminary trials, t-PA has been used in patients with thrombosed peripheral arteries and bypass grafts (6–9). The administration of t-PA in both human (6–9) and experimental animals (10–12) has been performed by intraarterial infusion proximal to the thrombus in the peripheral artery. The intravenous administration of t-PA also has a superior thrombolytic effect on the coronary thrombosis in humans (13) and in experimental animals (14–16). Whether or not the intravenous administration of t-PA has thrombolytic effects on peripheral thrombosis has not been determined.

Tissue culture urokinase (TCUK) was found in the culture medium of renal tissue, and it was demonstrated that TCUK was identical to urinary urokinase from the physico-chemical, enzymatic and immunological points of view (17, 18).

We attempted to elucidate whether the intravenous administration of t-PA could achieve arterial thrombolysis without systemic activation of the fibrinolytic system, as compared with TCUK, using a model in rabbits.

Materials and Methods

1. Materials and animals: Recombinant human tissue-type plasminogen activator (TD-2061, Lot No. R8605-1) was purified from the serum-free conditioned medium from a mouse cell line transformed with an expression vector designed for expression of t-PA cDNA, cloned from human uterine tissue, in our laboratory. One unit of rt-PA was compared with the WHO international
preparation of t-PA (83/517). Tissue culture urokinase (TCUK, Lot No. N657650) was purchased from Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan. $^{125}$I-labeled human fibrinogen (Lot No. 230) and human thrombin were purchased from Green Cross Corp., Osaka, Japan. Adult male rabbits, weighing 2 to 3 kg, were used.

2. Preparation of femoral artery thrombi in rabbits: Rabbits were anesthetized by giving them an intravenous injection of saline (1.5 ml/kg) containing pentobarbital sodium (19.2 mg/ml) and dimorpholamine (1.5 mg/ml). After administration of 0.5 ml of sodium iodide solution (0.5%, i.v.) and 0.2 ml atropine sulfate solution (0.05%, s.c.), the femoral artery was exposed through a 2 cm-dissection in the femoral region. The artery was isolated with two clamps over a distance of 1.5 cm up to the main bifurcation of the saphenous and femoral arteries. Small side branches were ligated the blood in this segment was washed out with saline, and then 40 $\mu$l of 0.5% sulfuric acid solution was injected into the lumen. This treatment caused injury on the intima in the artery to hold the thrombi formed in this position. After treatment of sulfuric acid for 2 min, the acid was washed out several times with saline. A 35 $\mu$l aliquot of homologous rabbit citrated blood was mixed with 5 $\mu$l of $^{125}$I-labeled human fibrinogen (300,000–400,000 cpm), human thrombin (0.025 U) and CaCl$_2$ (final conc. of 0.028 M). The clotting blood was quickly injected into the lumen of the femoral artery segments, and it was allowed to age for 25 min before both vessel clamps were removed.

3. Assessment of thrombolysis: Thrombolytic agents were infused through the contralateral marginal ear vein (systemically) or through the ipsilateral femoral artery (locally). Ten percent of the total dose of the thrombolytic agent was administered as a bolus loading dose, and the remaining 90% of the total dose was infused by a Harvard pump (model 975) at a rate of 0.3 ml/min (systemically) or 0.11 ml/min (locally) over a 0.5 hour period. One hour after terminating the infusion, the artery segment containing any residual clot was removed, and the radioactivity was measured in a gamma-counter.

Total radioactivity in the formed thrombus was calculated by subtracting the radioactivity remaining in the syringe and the catheter from the total radioactivity of the $^{125}$I-labeled fibrinogen used. The isotope recovery balance was made by calculating the radioactivity in the recovered thrombus, in the urine and in the blood at the end of the experiment, and was expressed as a percentage of the total radioactivity in the clot.

4. Assessment of systemic fibrinolysis: Blood samples (2.5 ml) were withdrawn using trisodium citrate (final conc. of 0.017 M) as the anticoagulant, before and one hour after terminating the infusion; and plasma was separated at 4°C by centrifugation for 5 min at 1,600 g and stored at −80°C until assay. Tranexamic acid (final conc. of 0.05 M) was added to the plasma to prevent fibrinogenolysis.

Plasma concentrations of $\alpha_2$-antiplasmin were evaluated by the chromogenic method, according to the instructions of the manufacturer (Daichi Pure Chemicals, Japan). Plasma concentrations of fibrinogen were determined according to Ratnoff and Menzie (19). The levels of fibrinogen and $\alpha_2$-antiplasmin were expressed as a percentage of the pre-infusion value.

5. Statistical analysis: Statistical significance was determined by Student's $t$-test.

Results

1. Thrombolytic activity of rt-PA: The thrombolytic effects of rt-PA and TCUK on the thrombus in the femoral artery are shown in Table 1. In groups treated with saline systemically and locally, the degree of thrombolysis was approximately 15% and 10%, respectively.

Although thrombolysis was not detected by systemic infusion of 100,000 IU/kg of rt-PA, 200,000 and 500,000 IU/kg of rt-PA produced a significant thrombolysis ($P<0.01$). In contrast to the case with rt-PA, systemic infusion of 200,000 IU/kg of TCUK induced non-significant thrombolysis. Local infusion of rt-PA resulted in significant thrombolysis at a dose as low as 500 IU/kg, while that of TCUK resulted in non-significant thrombolysis at a dose of 2,000 IU/kg.

These results indicate that rt-PA also produce a higher degree of thrombolysis than
Table 1. Extent of thrombolysis and isotope recovery after systemic and local infusion into rabbits with a femoral artery thrombosis

| Method of administration | Agents | Dose (I.U./kg) | N  | Thrombolysis(%) | Recovery (%) |
|--------------------------|--------|----------------|----|----------------|-------------|
| Systemic                | Saline | —              | 6  | 15.3 ± 1.90    | 101.0 ± 2.02|
|                         | rt-PA  | 100,000        | 6  | 13.1 ± 2.64    | 109.4 ± 7.44|
|                         | rt-PA  | 200,000        | 7  | 30.0 ± 3.26**  | 97.3 ± 3.57 |
|                         | rt-PA  | 500,000        | 7  | 50.0 ± 4.51**  | 95.8 ± 4.09 |
|                         | TCUK   | 200,000        | 6  | 21.4 ± 4.81    | 99.9 ± 2.00 |
| Local                   | Saline | —              | 7  | 10.4 ± 2.76    | 102.6 ± 2.35|
|                         | rt-PA  | 500            | 6  | 23.1 ± 5.29*   | 99.1 ± 3.41 |
|                         | rt-PA  | 2,000          | 7  | 39.0 ± 7.26**  | 110.3 ± 5.91|
|                         | rt-PA  | 5,000          | 6  | 61.0 ± 7.17*** | 106.0 ± 4.87|
|                         | TCUK   | 10,000         | 6  | 74.3 ± 4.55**  | 109.5 ± 8.75|
|                         | TCUK   | 2,000          | 4  | 16.5 ± 4.12    | 103.4 ± 2.22|
|                         | TCUK   | 10,000         | 6  | 49.0 ± 6.33**  | 96.4 ± 7.30 |

Each value represents the mean ± S.E. Statistically significant difference from the saline group: *P < 0.05, **P < 0.01.

Table 2. Effect of rt-PA and TCUK on α₂-antiplasmin and fibrinogen levels in rabbits with femoral artery thrombosis

| Agents | Dose (I.U./kg) | % of pre infusion value | α₂-antiplasmin | Fibrinogen |
|--------|----------------|-------------------------|----------------|------------|
|        |                |                         |                |            |
| Saline | —              | 97.5 ± 2.31             | 103.4 ± 2.46   |
| rt-PA  | 100,000        | 99.0 ± 3.53             | 105.4 ± 2.18   |
| rt-PA  | 200,000        | 94.8 ± 4.68             | 97.5 ± 1.28    |
| rt-PA  | 500,000        | 80.4 ± 3.12**           | 100.4 ± 1.97   |
| TCUK   | 200,000        | 52.7 ± 2.20**           | 99.9 ± 0.64    |

Data represent the mean ± S.E. (n=6–7) at one hour after the end of rt-PA or TCUK systemic infusion. Statistically significant difference from the saline group: **P < 0.01.

TCUK in local as well as systemic infusion, given in the same units. The isotope recovery balance exceeded 95% in each group.

2. Effect on plasma levels of fibrinogen and α₂-antiplasmin: In all experiments with systemic infusion of saline, rt-PA and TCUK, the plasma levels of fibrinogen and α₂-antiplasmin were measured one hour after terminating the infusion (Table 2). The fibrinogen level remained essentially unchanged in all groups. The administration of 200,000 IU/kg of rt-PA had no effect on the α₂-antiplasmin level, but administration of the same units of TCUK led to a significant decrease by about 50% in the α₂-antiplasmin level. With rt-PA, a slight decrease in α₂-antiplasmin was observed at a dose of 500,000 IU/kg.

Discussion

We examined thrombolysis and systemic fibrinolysis produced by systemic infusion of t-PA in rabbits with a femoral arterial thrombosis. In previously reported studies, dogs had been used as the experimental animal for preparing a femoral arterial thrombosis model (10, 12, 20, 21). However, these models do not serve routine purposes as it is difficult to use many dogs at one time and numerous materials are required for testing, especially in the case of systemic administration. To circumvent these problems, we developed an experimental artery thrombosis model in the rabbit femoral artery. The normal laboratory environment serves well for this purpose, and problems related to animal handling seem to
be minimized.

In this model, both locally and systemically administered rt-PA induced a marked thrombolysis in a dose-related manner. When rt-PA and TCUK were compared, in the same units, either local or systemic administration of rt-PA showed a higher degree of thrombolysis than TCUK. These results confirm the experiments performed in other laboratories in which t-PA was shown to be more effective than urokinase (2, 22–24).

At present, urokinase (UK) has been widely used as a conventional thrombolytic agent; however, a large amount of UK causes a hemorrhagic tendency due to non-specific fibrinolytic activation (25). Hence, in peripheral arterial occlusion, the clinical use of UK has been also limited to the more laborious intraarterial infusion technique (26, 27). On the other hand, it has been reported that the intravenous administration of t-PA can achieve successful thrombolysis in very rapid and safe fashion (28, 29). However, there have been few studies to compare the thrombolytic activity of t-PA by local administration with its activity by systemic administration in animals or humans with peripheral arterial thrombus.

In our model, local infusion of rt-PA resulted in significant thrombolysis at a lower dose than systemic infusion. This result agreed with the results obtained in the experimental vein thrombosis model reported by Collen et al. (2). Therefore, in peripheral thrombosis, it might be a major determinant for the thrombolytic process that t-PA was delivered in high concentration to the vicinity of a thrombus.

Certainly when thrombolytic agents are administered systemically, a large amount of them, as compared with local administration, seems to be required to obtain an adequate thrombolysis. In the present study, however, systemic administration of rt-PA resulted in a prompt dissolution of arterial thrombi without causing systemic activation of the fibrinolytic system, while TCUK induced a significant decrease in α₂-antiplasmin level without a significant thrombolytic effect. These findings are similar to those of other models of experimental thrombosis and implies that systemic administration of rt-PA will also lead to arterial thrombolysis with less danger of bleeding.

These results suggest that thrombolytic therapy with systemic administration of rt-PA warrants further attention for treating patients with thrombosed peripheral artery.

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