HEPARIN REQUIREMENT IN TISSUE-TYPE PLASMINOGEN ACTIVATOR-INDUCED EXPERIMENTAL CORONARY THROMBOLYSIS: COMPARISON WITH UROKINASE-INDUCED CORONARY THROMBOLYSIS

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The requirement of heparin in experimental coronary thrombolysis induced by tissue-type plasminogen activator (t-PA) was studied in closed-chest dogs with one hour old coronary thrombi and compared with that in urokinase (UK)-induced coronary thrombolysis. Animals were divided into 5 treatment groups as follows: group 1 received intracoronary t-PA alone (1,000 IU/kg/min; n = 5), and if thrombolysis was not induced within 40 to 50 min, dogs then received an intravenous injection of heparin (300 U/kg) plus intracoronary t-PA; group 2 received intravenous heparin at first, and if thrombolysis was not induced within 10 min, dogs subsequently received intracoronary t-PA (n = 5); group 3 also received intravenous heparin at first, and if thrombolysis was not induced within 10 min, dogs subsequently received t-PA but intravenously, as compared with the groups administered by the intracoronary route (n = 6); group 4 received intracoronary UK alone (1,000 IU/kg/min; n = 6); group 5 received intravenous heparin at first, and if thrombolysis was not induced within 10 min, dogs subsequently received intracoronary UK (n = 5). Thrombolysis was confirmed angiographically.

In group 1, coronary thrombolysis could not be induced within 44 ± 4 min by intracoronary t-PA alone, but it occurred in 8 ± 4 min when administered in combination with heparin in all dogs. Heparin alone failed to elicit reperfusion within 10 min in group 2, 3 and 5. t-PA, however, induced successful reperfusion in 16 ± 5 min (group 2) and in 23 ± 6 min (group 3), respectively.

Intracoronary urokinase alone elicited thrombolysis in 27 ± 4 min in group 4, but in group 5, treated in combination with heparin, rapid reperfusion was obtained in 12 ± 5 min (p < 0.001).

Key words:
t-PA
Thrombolysis
Heparin

(Received August 11, 1986; accepted November 27, 1986)
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This study was supported in part by grants (59770589, 60440109 & 60770632) from The Ministry of Education, Science, and Culture, a research grant for Cardiovascular Disease (61C-4) from The Ministry of Health and Welfare, and grants from Suzuken Memorial Foundation, and Mochida Memorial Foundation.
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t-PA did not lead to a significant degradation of fibrinogen, but UK decreased plasma fibrinogen significantly and heparin prevented the lowering of plasma fibrinogen and alpha₂-antiplasmin by reducing the required dosage of UK needed for recanalization.

The above results suggest that, in experimental t-PA-induced coronary thrombolysis, pre-administration of heparin is absolutely necessary. However, in urokinase-induced thrombolysis, heparin is not required for coronary reperfusion, due to the depletion of plasma fibrinogen.

The treatment of acute myocardial infarction with streptokinase (SK) or urokinase (UK) has been widely accepted, because the restoration of antegrade flow with thrombolytic agents can salvage substantial quantities of jeopardized myocardium. However, the use of these agents may reduce the level of circulating fibrinogen and possibly cause bleeding as an adverse effect.

Tissue-type plasminogen activator (t-PA) acts on clots and causes little or no bleeding. Therefore, it is expected to become a safer thrombolytic agent than SK or UK. It has recently been reported that the pre-administration of heparin enhances thrombolysis induced by SK and leads to the reduction of dosage of SK required to establish reperfusion.

However, the role of heparin in the thrombolysis with t-PA remains unclarified. In this study in dogs, we investigated the requirement of heparin for coronary thrombolysis by t-PA and compared with that by UK.

MATERIALS AND METHODS

Drugs: t-PA was supplied by Mochida Pharmaceutical Company Ltd., Tokyo, Japan. It was purified from the culture medium of a melanoma cell line. Heparin was obtained from Novo Industry Ltd., Copenhagen, Denmark.

Experimental preparation: Adult mongrel dogs (n = 31; 11 to 18 kg) were anesthetized with intravenous sodium pentobarbital (10 to 15 mg/kg) and received additional doses as required. Ventilation was maintained by a Harvard pump delivering room air through an endotracheal tube. A 8F Sones catheter was placed in the ascending aorta via the left carotid artery under fluoroscopy. A guide wire for coronary angioplasty (0.014 inch High-torque floppy, Advanced Cardiovascular System Inc., Temecula, California) was inserted through the catheter into the left anterior descending coronary artery. A copper coil (2 mm in length x 1 mm outside diameter) was advanced along the guide wire into the left anterior descending coronary artery, after which the guide wire was withdrawn. An occlusive thrombus formed within 5 to 10 min, and it was confirmed by coronary angiography using the contrast medium (Urographin 76%, Nihon Schering Corp., Osaka, Japan). Cineangiograms were taken on a cineangiographic system (WH-10, Shimadzu Company Ltd., Kyoto, Japan) equipped with a videographic apparatus (NV-9240X, Matsushita Electric Industrial Company Ltd., Osaka, Japan).

All of the animals studied developed electrocardiographic signs typical of ischemia. Four dogs were excluded from this study because of ventricular fibrillation during the one hour pre-treatment period. One hour after induction of coronary thrombus, the remaining 27 dogs were divided into 5 treatment groups as follows.

In group 1 (n = 5), t-PA was administered at a rate of 1,000 IU/kg/min by the intracoronary route through a catheter positioned in the left coronary ostium. If thrombolysis did not occur within 40 to 50 min, heparin (300 U/kg) was then injected intravenously and subsequently t-PA was administered in the same manner.

In group 2 (n = 5), the same dose of heparin as in group 1 was administered intravenously at first. If recanalization was not obtained within 10 min, t-PA was administered subsequently via the intracoronary route. In group 3 (n = 6), heparin was also administered at first, and if thrombolysis did not occur within 10 min, t-PA was administered but this time by the left external jugular vein.

In group 4 (n = 6), UK was administered at a rate of 1,000 IU/kg/min by the intracoronary route. In group 5 (n = 5), heparin was first administered, and if thrombolysis was not obtained within 10 min, UK was administered by the intracoronary route.

Continuous infusion of the thrombolytic agents was maintained by an infusion pump in all experiments. Additional dosage of heparin, other than mentioned above, was never used in this study.

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Thrombolysis was confirmed in each animal by coronary angiography performed at five-minute intervals or when evidence of reflow occurred, that is, at the onset of arrhythmias, or upon a decrease in ST segment elevation on the electrocardiographic tracings.

Measurement of fibrinogen levels and alpha₂-antiplasmin activities: For characterization of the extent of systemic activation of the fibrinolytic system, blood samples were drawn via a rear leg vein and mixed (9:1) with 3.8% W/V trisodium citrate before and after treatment. Samples were cooled immediately on ice and centrifuged. An 1-ml aliquot of the decanted plasma was mixed with 250 U/ml aprotinin (trasylool, the Bayer Co., Leverkusen, West Germany) to preclude proteolysis in vitro, and was stored at −20°C before the assay of fibrinogen. Another aliquot was frozen for the assay of alpha₂-antiplasmin. The lowest detectable limit was 25%.5

Statistical analysis: All data were expressed as mean ± standard deviation. Data were compared using paired or unpaired t-test. A p value of less than 0.05 was considered significant.

RESULTS

t-PA (1,000 IU/kg/min) alone administered by the intracoronary route for 44 ± 4 min did not induce thrombolysis in any dog in group 1. A subsequent heparin (300 U/kg) injected intravenously with infusion of t-PA caused thrombolysis 8 ± 4 min later in all dogs (Fig. 1).

Heparin alone failed to induce thrombolysis within 10 min in both group 2 and 3, but when the dogs received t-PA (1,000 IU/kg/min) by the intracoronary route (group 2) or by the intravenous route (group 3) thrombolysis was obtained in 16 ± 5 min and 23 ± 6 min, respectively.

t-PA did not lead to a significant depletion of fibrinogen (group 1; from 305 ± 27 mg/dl to 280 ± 28 mg/dl, group 2; from 315 ± 35 mg/dl to 285 ± 36 mg/dl, group 3; from 305 ± 30 mg/dl to 293 ± 20 mg/dl), but it lowered alpha₂-antiplasmin significantly (group 1; from 112.4 ± 4.7% to 79.0 ± 10.8%, p < 0.001, group 2; from 114.2 ± 5.5% to 96.4 ± 14.2%, p < 0.05, group 3; from 108.7 ± 8.7% to 95.2 ± 14.0%, p < 0.05). Alpha₂-antiplasmin value after t-PA infusion did not show statistically significant differences in the 3 groups; but the level in group 1, who received the longest infusion of t-PA, was the lowest in the 3 groups.

In group 4, intracoronary UK alone elicited successful reperfusion in 27 ± 4 min. In group 5, heparin also failed to induce reperfusion within 10 min, but additional intracoronary UK induced thrombolysis rapidly in 12 ± 5 min (p < 0.001).

UK decreased fibrinogen significantly from 314 ± 29 mg/dl to 90 ± 35 mg/dl in group 4 (p < 0.001) and from 299 ± 27 mg/dl to 200 ± 26 mg/dl in group 5 (p < 0.001). UK also decreased alpha₂-antiplasmin significantly from 113.2 ± 38% to the lowest detectable level in group 4 (p < 0.001) and from 110.2 ± 8.2% to 48.0 ± 22.2% in group 5 (p < 0.001). Heparin

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also diminished the degradation of plasma fibrinogen (90 ± 35 mg/dl in group 4 vs 200 ± 26 mg/dl in group 5, p < 0.001) and alpha_2-antiplasmin (lowest detectable limit in group 4 vs 48.0 ± 22.2% in group 5, p < 0.001).

DISCUSSION

In this study, we investigated the role of heparin in thrombolysis with t-PA. t-PA via the intracoronary or intravenous route induced thrombolysis after pretreatment with heparin. These results suggest that 1,000 IU/kg/min of t-PA is enough to induce coronary thrombolysis even when administered intravenously with heparin pre-administration in this model.

However, 1,000 IU/kg/min of t-PA alone administered by the intracoronary route failed to lyse the coronary thrombus, and the addition of heparin caused thrombolysis. Heparin alone failed to elicit reperfusion in this model. Thus, our findings suggest that heparin is essential for thrombolysis with t-PA in this model.

In contrast, UK induced coronary thrombolysis without heparin pretreatment, and the addition of heparin reduced the dosage of UK required for thrombolysis.

Alpha_2-antiplasmin activity was decreased by t-PA. This indicates that, at the dosage used, t-PA induces some degree of activation of plasma plasminogen. The magnitude of this effect, however, was less than that of UK. Thus, t-PA did not lead to a significant depletion of fibrinogen even when administered two times longer than the time required for successful thrombolysis with heparin pre-administration. In contrast, UK decreased fibrinogen significantly, and heparin reduced the dosage of UK required for recanalization, which prevented the lowering of plasma fibrinogen levels.

Some studies have found a high correlation between a systemic fibrinolytic state (decrease in plasma fibrinogen) induced by SK and recanalization. Others have reported that heparin enhances SK-induced thrombolysis in animal preparation. It is well known that the degradation of fibrinogen or a large dosage of heparin impairs coagulation and predisposes to systemic bleeding, as well as inhibits the new fibrin formation. These findings suggest that the formation of coronary thrombus may represent a balance between new fibrin deposition and fibrin degeneration, and it seems that the prevention of new fibrin deposition is an important requirement for the successful thrombolysis as augmentation of fibrin degeneration.

From this point of view, these mechanisms may relate to the frequent reocclusion in successful thrombolysis with t-PA, because its half-life is short.

We did not examine whether higher doses of t-PA than the dose being applied in this study could induce the coronary thrombolysis in this model. However, intracoronary t-PA with the above-mentioned dose did not induce thrombolysis as shown in our study, and a higher dosage of t-PA has the systemic effects with significant reduction of plasma fibrinogen levels.

A recent report suggested that heparin was one of the major causes of bleeding complications and t-PA alone would induce the successful thrombolysis with infrequent major bleeding. When a higher dosage of t-PA is employed, successful thrombolysis without heparin may be possible, but it will result in the significant reduction of plasma fibrinogen.

Thus, t-PA may indeed be an effective intravenous thrombolytic drug, but heparin seems to be a necessary requirement for the successful thrombolysis by t-PA with the dose of safety range.

Acknowledgement

We express our appreciation to Daniel Mrozek for help with the preparation of the manuscript.

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