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Thrombolysis by human tissue plasminogen activator and urokinase in rabbits with experimental pulmonary embolus

O. Matsuo, D. C. Rijken & D. Collen

Center for Thrombosis and Vascular Research, Department of Medical Research, University of Leuven, Belgium

Thromboembolism is a frequent and often lethal complication of many medical diseases and surgical procedures. Streptokinase, a bacterial non-enzymatic protein, and urokinase, an enzyme obtained from human urine, are both potent activators of the human fibrinolytic system, and have been extensively investigated as a means of medical treatment of this condition. Although it is well established that these agents can clear obstructed vessels in roughly 50% of cases, the fact that they induce relatively extensive systemic fibrinogen breakdown and a serious haemorrhagic diathesis has prevented their general application for thrombolysis. The physiological plasminogen activator in blood (blood activator), which is probably released from the vascular wall (vascular activator) and is identical or at least very similar to the activator extracted from human organs (tissue activator), differs from urokinase (for references see ref. 1). This activator has a markedly higher fibrinolytic to fibrinogenolytic ratio than urokinase *in vitro*[1] and might therefore constitute a better thrombolytic agent. We have developed a purification method for tissue plasminogen activator from a human melanoma cell line which enables us to produce milligramme quantities on a laboratory bench scale[2] and we have now compared its thrombolytic effect with that of urokinase in an experimental animal model. We have found that tissue plasminogen activator causes thrombolysis at lower doses than urokinase, without extensive plasminogen activation in the circulating blood and without haemostatic breakdown.

The fibrinolytic activities of tissue plasminogen activator and urokinase were determined on plasminogen-enriched bovine fibrin plates[3] and expressed in International Units (IU) using the WHO 1st International Reference Preparation of urokinase as described previously[4]. The specific activities of both enzymes were very similar to those described elsewhere[2]. Human fibrinogen was prepared as described by Blombäck and Blombäck[5] and labelled with ^{125}I according to McFarlane[6].

An experimental thrombus was produced in a polyethylene tube of 3 mm internal diameter by mixing citrated whole human blood (0.5 ml) with 8 μl of ^{125}I-labelled human fibrinogen (~10^6 c.p.m.), 50 μl of 25 mM $CaCl_2$ and 2 μl of human thrombin (final concentration 1 NIH unit per ml). After incubation at room temperature for 15 min, 1.5 cm length of the tube (~0.10 ml thrombus) was cut out of the middle position of the tube. The thrombus was poured into a Petri dish, washed repeatedly with 0.15 M NaCl and its ^{125}I content measured. The radioactive thrombus was then aspirated in a silicon catheter for injection.

New Zealand White rabbits were anaesthesized with 0.5 ml Hypnorm and 0.3 ml pentobarbital. A jugular vein was dissected free, cut over a distance of 3 mm and, after injection of the thrombus, carefully sutured to restore the blood flow. The activator infusions (tissue plasminogen activator or urokinase) were given intravenously into a contralateral marginal ear vein as a bolus injection of 10% of the total dose followed by a continuous infusion over 6 or 12 h as indicated; blood samples were collected from a femoral vein catheter on citrate at regular time intervals for up to 24 h. The animals were killed 9 or 24 h after the start of the infusion, the thorax was opened, the lungs and heart removed together and the thrombus localized with the use of an isotope monitor. The lungs were then carefully dissected, the pulmonary artery incised and the remaining radioactive thrombus recovered. The degree of thrombolysis was calculated as the difference in ^{125}I content of the injected and recovered thrombus. In all but three instances the remaining thrombus was recovered as a single fragment, tightly lodged in a major branch of the pulmonary artery.

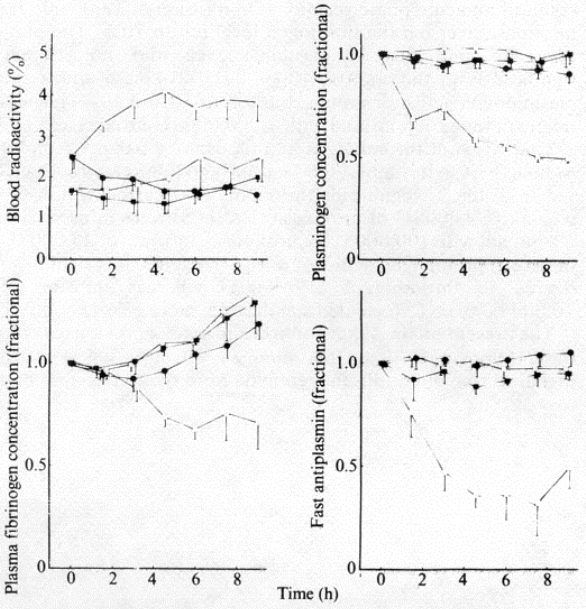

Fig. 1 Changes in the main blood parameters during infusion of tissue plasminogen activator or urokinase in rabbits. ■, Control animals infused with saline; ●, 100,000 units of urokinase in five rabbits; □, 1,000,000 units of urokinase in three rabbits; ○, 35,000 units of tissue plasminogen activator over 6 h in six rabbits and 70,000 units over 12 h in three rabbits. The data represent mean values ±s.d. Blood radioactivity was expressed as per cent of the injected radioactivity present in the total blood volume.

Figure 1 represents changes in the main blood parameters during the first 9 h of the infusion. The radioactivity in the blood rose to ~2% of the amount injected in samples taken after 15 min. In the control groups without activator infusion and in the urokinase groups the blood radioactivity remained at this level throughout the experiment, whereas infusion of tissue plasminogen activator (35,000 IU over 6 h or 70,000 IU over 12 h) resulted in a significant rise in blood radioactivity (from 2.2% to a maximum of 4.1%). The fibrinogen level[5] rose progressively in controls and in those given 10,000 IU urokinase tissue plasminogen activator (infused over 6 h), probably as a

Table 1 Extent of thrombolysis and isotope recovery

Group	*n*	% Thrombolysis	% Recovery
Saline 9 h	4	3.2±1.5	102.0±2.4
Saline 24 h	6	5.8±3.2	100.3±4.4
UK 100,000	5	4.0±1.2	103.3±1.1
UK 1,000,000	3	11.5±5.4	97.7±2.9
TA 35,000	6	16.5±2.7	100.4±3.3
TA 70,000	3	23.1±7.4	97.0±0.8

The extent of thrombolysis was determined as the difference in radioactivity between the injected and recovered clots. The isotope recovery was measured as the sum of the radioactivity present in the recovered thrombus, the blood, the urine and the lungs and expressed as per cent of the injected tracer. UK, urokinase; TA, tissue plasminogen activator; *n*, number of experimental animals. The data represent means ± s.d.

result of an acute phase reaction. On infusion of 1,000,000 IU urokinase over 6 h the fibrinogen level fell to 70%. The plasminogen[7] and fast antiplasmin[8] levels did not change significantly in the control, 100,000 IU urokinase and tissue plasminogen activator groups, but fell to 50 and 30%, respectively, in the group infused with 1,000,000 IU urokinase.

The extent of thrombolysis and the isotope balance (sum of radioactivity in thrombus, blood, urine and lungs) are represented in Table 1. Significant thrombolysis occurred within 9 h when 1,000,000 IU of urokinase ($P<0.05$) were infused over 6 h but not with 100,000 IU of urokinase. Infusion of 35,000 IU of tissue plasminogen activator over 6 h resulted in a significant degree of thrombolysis ($P<0.001$) whereas infusion of 70,000 IU over 12 h yielded significantly more lysis ($P<0.01$).

The present findings thus confirm and extend previous *in vitro* observations that tissue plasminogen activator has a much higher fibrinolytic to fibrinogenolytic ratio than urokinase and suggest that it might have greater potential as a thrombolytic agent.

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