ENHANCEMENT OF CORONARY THROMBOLYSIS WITH PLASMINOGEN PRO-ACTIVATOR BY PRETREATMENT WITH HEPARIN

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The effect of pretreatment with heparin on coronary thrombolysis by plasminogen pro-activator was studied in 20 dogs with a 1-h old clot in the left anterior descending coronary artery. The clot was induced by placement of a copper coil and thrombolysis was confirmed angiographically. Intravenous administration of plasminogen pro-activator at a rate of 5 $\mu\text{g/kg/min}$ (group 1; $n = 5$) and 20 $\mu\text{g/kg/min}$ (group 2; $n = 5$) after pretreatment with heparin (300 IU/kg) induced thrombolysis in 31 ± 4 min and 14 ± 2 min, respectively. When plasminogen pro-activator was administered intravenously at a rate of 20 $\mu\text{g/kg/min}$ without heparin pretreatment (group 3; $n = 5$), the infusion interval for successful thrombolysis was prolonged to 45 ± 6 min, which was significantly longer than that in groups 1 and 2 ($p < 0.001$). In these three treatment groups, thrombolysis was not associated with severe alteration in plasma hemostatic factors (fibrinogen and α_2 -antiplasmin). An evaluation of plasma urokinase activities using a chromogenic substrate S-2444 did not show that heparin increased the plasma urokinase activities. By increasing the dose of plasminogen pro-activities. By increasing the dose of plasminogen pro-activator to 80 $\mu\text{g/kg/min}$, successful reperfusion was rapidly obtained in 25 ± 5 min without heparin pretreatment (group 4; $n = 5$); this was significantly faster than the results seen in group 3 ($p < 0.001$). An analysis of urokinase activities showed that plasminogen pro-activator was fully converted to urokinase, which induced complete depletion of fibrinogen and α_2 -antiplasmin. These results suggest that heparin can enhance coronary thrombolysis in dogs and the mechanism of the enhancement is not related to the plasma urokinase activities. In addition, heparin may reduce the risk of the depletion of plasma fibrinogen by lowering the doses of plasminogen pro-activator necessary for reperfusion.

THE treatment of acute myocardial infarction with streptokinase or urokinase has been widely accepted, because the restoration of anterograde flow with thrombolytic agents can salvage substantial quantities of jeopardized

myocardium.¹⁻³ However, the use of these agents may reduce the level of circulating fibrinogen and possibly cause bleeding as an adverse effect. For this reason, several attempts have been made to develop fibrin-specific thrombolytic agents for safer but still effective thrombolysis.

Plasminogen pro-activator (pro-urokinase, or single chain, urokinase-type plasminogen acti-

Key words:

Plasminogen pro-activator
Coronary thrombolysis
Heparin

(Received June 18, 1987; accepted August 5, 1987)

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vator) is one of the fibrin-specific thrombolytic agents currently under investigation. It is an inactive precursor of urokinase which is activated by conversion to the two chain form in the presence of plasmin. Under experimental conditions, its activation is limited to the site of a clot⁴ and several reports have confirmed its clot-selective thrombolytic effect in animal preparations^{5,6}

It has recently been reported that the pre-administration of heparin enhances thrombolysis induced by streptokinase and leads to a reduction in the dose of streptokinase required to establish reperfusion.⁷ However, the role of heparin in thrombolysis with plasminogen pro-activator remains to be determined. If rapid thrombolysis with plasminogen pro-activator can be induced by pretreatment with heparin, both a reduction in myocardial damage and an improvement in the safety of thrombolysis can be expected.

Accordingly, the purpose of this study was to investigate whether coronary thrombolysis with plasminogen pro-activator could be enhanced by pretreatment with heparin.

METHODS AND MATERIALS

Drugs: Plasminogen pro-activator was supplied by Green Cross Corp. Ltd., Osaka, Japan. It was purified from the culture medium of a human kidney cell line. The material could be fully activated by plasmin and displayed a specific activity of 150,000 IU/mg protein by a colorimetric assay with the synthetic substrate S-2444.⁸

Heparin was obtained from Novo Industry Ltd., Copenhagen, Denmark.

Experimental preparation: Adult mongrel dogs (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. An 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 $\times$ 1 mm outside diameter) was advanced along the guide wire into the left anterior descending coronary artery, and then the guide wire was withdrawn. An occlusive

thrombus formed within 5–10 min^{9,10} confirmed by coronary angiography using contrast medium (Urographin 76%, Nihon Schering Corp., Osaka, Japan). Cineangiograms were taken on a cineangiographic system (WHA-10, Shimadzu Company Ltd., Kyoto, Japan) equipped with a videographic apparatus (NV-9240X, Matsushita Electric Industrial Company Ltd., Osaka, Japan). One hour after induction of the coronary thrombus, total occlusion was confirmed angiographically in each dog, and then, plasminogen pro-activator was infused via a peripheral vein as follows.

In group 1 ($n = 5$), plasminogen pro-activator was administered at a rate of 5 μ g/kg/min with preadministration of heparin (300 U/kg). In group 2 ($n = 5$), plasminogen pro-activator was administered at a rate of 20 μ g/kg/min with preadministration of the same dose of heparin. In group 3 ($n = 5$), plasminogen pro-activator was administered at a rate of 20 μ g/kg/min without preadministration of heparin. In group 4 ($n = 5$), plasminogen pro-activator was administered at a rate of 80 μ g/kg/min without heparin preadministration.

Thrombolysis was confirmed in each animal by coronary angiography performed at 5 min 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. In this study, heparin was not administered for the prevention of possible thrombosis caused by the catheter or the guide wire, and the catheter was re-inserted each time for angiography.

Assessment of the fibrinogen level and α_2 -antiplasmin activity: Blood sample were collected in citrate before and at 15 min intervals after the onset of plasminogen pro-activator infusion and at the time when reperfusion was confirmed in groups 1, 2 and 3. In group 4, blood sampling was performed before and 5, 10, 20, and 30 min after the onset of plasminogen pro-activator infusion. Samples were cooled immediately on ice and centrifuged. Plasma samples were frozen at -70°C before the analysis of fibrinogen¹¹ and α_2 -antiplasmin activity.¹²

Assessment of urokinase activity: Urokinase activities in the plasma samples were determined by a colorimetric assay with the synthetic substrate S-2444 (Kabi Diagnostics, Stockholm, Sweden) in a buffer solution containing 0.05 mol/l tris-HCl, pH 8.8, 0.038 mol/l NaCl and 0.2% bovine serum albumin. An aliquot of

Fig.1. Left coronary arteriogram (left lateral projection) after introduction of the copper coil (upper) and after successful thrombolysis (lower) with plasminogen pro-activator. Arrows indicate the copper coil in the anterior descending coronary artery.

plasma was preincubated with plasmin (1.6 CU/ml) for 30 min at 37°C in the buffer solution. After the addition of aprotinin (200 KIU/min) in order to quench the plasmin, urokinase activity was determined by a colorimetric assay. Under these conditions, plasminogen pro-activator was shown to cause full conversion to the two chain form⁸. Thus, this urokinase activity is defined as the potential urokinase activity in the plasma. In the other aliquot of plasma, urokinase activity was also determined but without preincubation with plasmin. This urokinase activity is defined as the actual urokinase activity.

Statistical analysis: All data were expressed as mean $\pm$ standard deviation. Data were compared using a paired or unpaired t-test and multiple comparisons with one way analysis of variance. A p value of less than 0.05 was con-

Fig.2. Infusion intervals of plasminogen pro-activator necessary for reperfusion in each experiment. Data are mean $\pm$ SD. *p < 0.005, **p < 0.001; unpaired t test. PPA = plasminogen pro-activator.

sidered significant.

RESULTS

With heparin pretreatment, 5 μ g/kg/min of plasminogen pro-activator (group 1) induced angiographic reperfusion in 31 ± 4 min (Fig. 1) and 20 μ g/kg/min of plasminogen pro-activator (group 2) induced rapid reperfusion in 14 ± 2 min (p < 0.001, Fig. 2).

Without heparin pretreatment, 20 μ g/kg/min of plasminogen pro-activator could lyse the thrombus in 45 ± 6 min (group 3), but the infusion interval was longer than that of the dogs in groups 1 and 2 (p < 0.001). A higher dose of plasminogen pro-activator (80 μ g/kg/min; group 4) could reduce the infusion interval to 25 ± 5 min (p < 0.001), but this was still longer than that of the dogs in group 2 (p < 0.001).

As shown in Table I, infusion of 5 μ g/kg/min or 20 μ g/kg/min of plasminogen pro-activator did not induce significant changes in the fibrinogen level. The α_2 -antiplasmin activity was not

TABLE 1 CHANGES OF FIBRINOGEN, ALPHA₂-ANTIPLASMIN, AND UROKINASE ACTIVITIES

Group/Infusion interval	Fibrinogen (%)	Alpha ₂ -antiplasmin (%)	Urokinase activity (IU/ml)	
			without plasmin	with plasmin
Group 1				
control	100	100	1.2 ± 1.1	12.0 ± 3.3
15 min	100 ± 5	98 ± 5	5.0 ± 1.0	122 ± 19
31 ± 4 min	95 ± 7	95 ± 6	6.8 ± 2.6	162 ± 31
Group 2				
control	100	100	0.6 ± 0.5	10.8 ± 3.8
14 ± 2 min	100 ± 4	99 ± 2	12.4 ± 9.4	539 ± 69
Group 3				
control	100	100	1.2 ± 0.4	13.0 ± 2.3
15 min	96 ± 6	98 ± 4	8.2 ± 3.3	548 ± 40
30 min	95 ± 6	95 ± 3	31.6 ± 17.6	603 ± 87
45 ± 6 min	98 ± 5	92 ± 5*	28.2 ± 15.3	636 ± 91
Group 4				
control	100	100	0.4 ± 0.5	11.4 ± 3.0
5 min	98 ± 6	93 ± 3	12.2 ± 6.7	593 ± 23
10 min	91 ± 9*	61 ± 18**	50.2 ± 29.5	1156 ± 97
20 min	0**	0**	1670 ± 93	1625 ± 95
30 min	0**	0**	1927 ± 118	1990 ± 104

The values of the fibrinogen and alpha₂-antiplasmin as expressed as the fraction of the values before the start of plasminogen pro-activator infusion. without plasmin = the actual urokinase activity; with plasmin = the potential urokinase activity; *p < 0.05, **p < 0.001 (multiple comparisons) compared with the control value.

changed in groups 1 and 2 during thrombolysis. In group 3, however, it was significantly decreased when reperfusion occurred ($p < 0.05$). In group 2, when reperfusion was obtained, the fibrinogen level and alpha₂-antiplasmin activity were not significantly different from those of group 3 at the 15 min interval.

As shown in Fig. 3, the potential urokinase activity increased during the infusion of plasminogen pro-activator in groups 1, 2, and 3. The actual urokinase activity also increased during thrombolysis. The potential urokinase activity was over 10 times higher than the actual urokinase activity in all plasma samples. In group 2, when reperfusion was obtained, both the potential urokinase activity and the actual urokinase activity were not different from those of group 3 at the 15 min interval.

In group 4, however, fibrinogen and alpha₂-antiplasmin decreased rapidly and were completely depleted when reperfusion was obtained. These results were consistent with the changes in

the actual plasma urokinase activity, which increased to the level of the potential urokinase activity as shown in Fig. 4. Within 10 min after the onset of plasminogen pro-activator infusion, the fibrinogen and alpha₂-antiplasmin were $91 \pm 9\%$ and $61 \pm 18\%$ of the control value, respectively. The actual urokinase activity was also within 10% of the potential urokinase activity, which was consistent with the results of groups 1, 2, and 3. With infusion of $80 \mu\text{g/kg/min}$ of plasminogen pro-activator for more than 20 min, however, fibrinogen and alpha₂-antiplasmin declined to zero with a rapid increase of the actual urokinase activity to the level of the potential urokinase activity in the plasma. Therefore, the degradation of fibrinogen and alpha₂-antiplasmin which occurred within 10 min was induced by the urokinase which was contaminating the plasminogen pro-activator. However, the degradation observed in 20 min was induced by the full activation of plasminogen pro-activator in the plasma.

Fig. 3. Plasma urokinase activities during intravenous infusion of plasminogen pro-activator. ●▲ represent the potential urokinase activities in the plasma, which were determined after plasmin incubation. ○□ represent the actual urokinase activities in the plasma, which were determined without plasmin incubation. ●○ = group 1, ▲△ = group 2, ■□ = group 3.

DISCUSSION

The results of this study confirm that pretreatment with heparin can enhance the thrombolytic effect of plasminogen pro-activator. This observation is consistent with our previous report that the preadministration of heparin can strongly augment the thrombolytic efficacy of tissue-type plasminogen activator in dogs.¹⁰ These findings in clot-selective thrombolytic agents have also been observed in the conventionally used thrombolytic agents, streptokinase and urokinase. In experimental coronary thrombolysis, heparin pretreatment allows a significant reduction of the dose of urokinase necessary for reperfusion¹⁰ and increases the success rate of streptokinase-induced thrombolysis.⁷ In patients with pulmonary emboli, heparin also reduces the necessary dose of streptokinase¹³ or urokinase.¹⁴ These findings suggest that heparin enhances the efficacy of thrombolytic agents.

Our study also shows that heparin does not

Fig. 4. Changes in fibrinogen, α_2 -antiplasmin activity, and urokinase activities in group 4. Upper panel; changes in fibrinogen. Middle panel; changes in α_2 -antiplasmin. Data are expressed as the fractional changes of the control values for each factor. * $p < 0.05$, ** $p < 0.001$ (multiple comparisons) compared with the values before the start of plasminogen pro-activator infusion. Lower panel; changes in urokinase activities. ● represents the potential urokinase activity and ○ represents the actual urokinase activity. Results are mean $\pm$ SD. ** $p < 0.001$ (paired t-test).

increase the actual urokinase activity or the potential urokinase activity. Heparin has been shown to stimulate the release of tissue-type plasminogen activator,¹⁵ but the level of the released tissue-type plasminogen activator was negligible in therapeutic doses.¹⁶ Heparin is known to have no direct fibrinolytic activity.¹⁷ Thus, the mechanism of enhancement is not the augmentation of fibrinolytic activity, but may be the blocking of formation of new fibrin and its incorporation into the thrombus during thrombolytic therapy as shown in reports from other laboratories.^{18,19}

Plasminogen pro-activator at the rate of 80 $\mu\text{g}/\text{kg}/\text{min}$ induced depletion of fibrinogen and

alpha₂-antiplasmin when reperfusion was obtained. This was the result of the activation of plasminogen pro-activator to the two-chain form in the plasma. A recent report has shown that in plasma incubated with 500 to 1,000 IU/ml of plasminogen pro-activator, plasmin and urokinase are generated spontaneously and simultaneously after incubation for 1–3 h.⁴ The dose of plasminogen pro-activator needed to induce spontaneous conversion in humans remain to be investigated. But these findings suggest that a spontaneous conversion of plasminogen pro-activator to the two-chain form may be induced by a higher dose of plasminogen pro-activator.

The degradation of fibrinogen is also beneficial for thrombolysis. Cercek et al. reported that pretreatment with ancrod produced a quantitatively similar enhancement of the thrombolytic effect of recombinant tissue-type plasminogen as did pretreatment with heparin in dogs with arterial thrombi.¹⁸ In patients with acute myocardial infarction, some studies have found a strong correlation between the depletion of fibrinogen induced by streptokinase and recanalization.^{20,21} Collen et al. also reported that fibrinogen breakdown was significantly higher in patients with successful recanalization than in unresponsive patients treated with recombinant tissue-type plasminogen activator.²² It is not clear whether the depletion of fibrinogen in the dogs in group 4 contributed to the thrombolysis, but from the view point of the urokinase activity (the activity of plasminogen pro-activator is 150,000 IU/mg protein), it is wasteful, because urokinase has been shown to recanalize the same type of thrombus at a lower dose^{6,23} and complete depletion of fibrinogen may cause serious bleeding. Our data suggest that heparin can also reduce the risk of the complete depletion of fibrinogen by reducing the dose of plasminogen pro-activator necessary for reperfusion.

Clinical implications: This study suggests that heparin can enhance the thrombolytic efficacy of plasminogen pro-activator. When a higher dose of plasminogen pro-activator is employed, successful thrombolysis may be possible, but it will result in a significant reduction of plasma fibrinogen by either the urokinase contaminating the plasminogen pro-activator or the urokinase spontaneously generated. The depletion of fibrinogen also enhances the thrombolysis, but it is undesirable because the recovery of fibrinogen is not rapid. On the other hand, the effect of heparin is readily reversible. Thus, the use of

heparin seems preferable.

Acknowledgment

We express our appreciation to Barbara Chaffee, M.D. for her help with the preparation of the manuscript.

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