Early Thrombolysis by Recombinant Tissue-Plasminogen Activator Is Beneficial to the Ischemic Myocardium

Katsuya HIGO, Akira KARASAWA, and Kazuhiro KUBO

Department of Pharmacology, Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Suno-gun, Shizuoka 411, Japan

(Received June 23, 1991)

We examined the effect of coronary thrombolysis by recombinant tissue-plasminogen activator (rtPA) on infarct size using a thrombin-induced thrombosis model of open-chest anesthetized dog. Occlusive thrombus was induced by injection of thrombin (100 U) in the left anterior descending coronary artery (LAD). The intravenous infusion of rtPA (10 μg/kg/min) was started at 30 min (30 min-ischemia group) or at 60 min (60 min-ischemia group) after the formation of thrombus, and was continued for 30 min. Spontaneous thrombolysis was not observed in the 360 min-ischemia (vehicle-treated) group. Intravenous infusion of rtPA elicited thrombolysis within 30 min in all the dogs except in one in the 60 min-ischemia group. The infarct size was significantly reduced by rtPA-induced thrombolysis. The shorter the duration of the ischemia, the longer the effect of the drug, and the infarct size after thrombolysis was smaller in the 30 min-ischemia group than in the 60 min-ischemia group. Ischemia-induced changes in ST-segment of electrocardiogram (ECG) were significantly ameliorated after thrombolysis in both 60 min- and 30 min-ischemia group. These results suggest that early reperfusion of coronary thrombosis by rtPA is beneficial to the ischemic myocardium.

Keywords — recombinant tissue-plasminogen activator; thrombosis model; infarct size; thrombolysis; myocardial infarction; electrocardiogram

Introduction

Recombinant tissue-plasminogen activator (rtPA) is a well established thrombolytic agent which is employed in the treatment of patients with acute myocardial infarction. rtPA has strong affinity for fibrin\(^1\) and thus induces thrombolysis with minimal systemic lytic effect even by intravenous infusion, whereas urokinase and streptokinase have strong systemic lytic effects, which result in a bleeding tendency when they are administered from peripheral vessels.

Because myocardial necrosis is caused by lack of oxygen supply via coronary arteries, recanalization of the obstructed coronary arteries is assumed to result in the salvage of jeopardized myocardium and in the reduction of infarct size. On the other hand, it has been suggested that coronary recanalization may do harm to the functional restoration of the reperfused myocardium via mechanisms involving intramyocardial hemorrhage\(^2\) as well as generation of oxygen-derived free radicals\(^3\) associated with reperfusion. Thus, coronary reperfusion could exacerbate rather than salvage the jeopardized myocardium.

The present study was designed to determine whether early reperfusion by thrombolysis is beneficial to jeopardized myocardium or not, in an anesthetized canine model of thrombin-induced coronary thrombosis.

Materials and Methods

1. Experimental Preparation — Adult mongrel dogs of either sex (9.4—19 kg) were anesthetized with pentobarbital sodium (30 mg/kg, i.v.), intubated and ventilated with room air. The right femoral artery and vein were exposed and catheters were inserted for monitoring arterial blood pressure (AP-621G, Nihon Kohden) and for administration of drugs, respectively. Small stainless steel needle electrodes were placed in the right forelimb and the left hindlimb for the recording of electrocardiogram (ECG) (lead II) (AT-601G, Nihon Kohden). Heart rate was monitored by triggering from ECG (AT-601G, Nihon Kohden). All parameters were recorded on a recorder (WS-681G, Nihon Kohden).

A left thoracotomy was performed at the fifth intercostal space and the heart was exposed and suspended in a pericardial cradle. A 10 to 15 mm
section of the left anterior descending coronary artery (LAD) was isolated for thrombus formation. An electromagnetic flow probe was placed at the proximal end of the isolated LAD for measuring coronary artery blood flow (MEV-3100, Nihon Kohden).

2. Experimental Protocol — After a 30-min stabilization period, a thrombus was induced in the LAD between the first and the second diagonal branch by securing both the proximal and the distal snare ligatures and injecting thrombin (50—200 U) (Green Cross). The proximal ligature was slightly released and tightened repeatedly so as to mix the fresh blood with thrombin. Seven minutes after the proximal ligature was secured, this same ligature was released, and after an additional 3—8 min, the distal ligature was released. The formation of thrombus was confirmed by the absence of coronary blood flow as measured by the electromagnetic flow meter.

At 30 min (30 min-ischemia group) or 60 min (60 min-ischemia group) after the formation of thrombus, sodium heparin (300 U/kg) was administered intravenously and rtPA (10 μg/kg/min) was infused for 30 min via a catheter inserted into the right femoral vein. The dose of rtPA was determined in the preliminary study. In fact, at a dose of 5 μg/kg/min of rtPA, thrombolysis was not achieved within 30 min of infusion in all 3 dogs examined, while at a dose of 10 μg/kg/min, thrombolysis occurred in all the dogs within 30 min. Dogs in the 360 min-ischemia group received the vehicle for rtPA (excipient) for 30 min starting at 30 min after the formation of thrombus. During LAD occlusion and reperfusion, animals received lidocaine (Xylocaine® , Fujisawa-Astra) to minimize fatal arrhythmia occurring. High speed tracings were taken every 30 min during the experiment and the changes in ST-segment elevation or decline were measured.

Twenty one dogs were used in the present study. In the 360 min-ischemia group, 1 out of the 6 dogs died during the occlusion period because of ventricular fibrillation. Three of the 8 dogs in the 60 min-ischemia group and 2 of the 7 dogs in the 30 min-ischemia group died of ventricular fibrillation during the occlusion or reperfusion period. The remaining 15 dogs, 5 in each group, were used for analysis.

3. Measurement of Infarct Size — The area at risk and the infarcted area were delineated by a dual staining technique adopted by Toki et al. At 360 min after the formation of thrombus, 20 ml of arterial blood was collected in a test tube containing sodium heparin (5 U/ml). The cannula was inserted retrogradely into the second diagonal branch and the heparinized whole blood was infused at mean aortic blood pressure. After the distal ligature was reoccluded, 20 ml of Evans’ blue dye solution (0.1 g/ml in 50 mM phosphate buffered saline, pH 7.4) was injected into the right femoral vein, making the normal (non-ischemic) area blue. The area at risk remained unstained because of the infusion of undyed blood. The dog was killed with an intravenous injection of potassium chloride and the heart was removed. The whole heart was suspended in saline in a water bath and the risk area of the heart was perfused with a solution consisting of 0.2% 2,3,5-triphenyltetrazolium chloride (TTC) in 50 mM phosphate-buffered saline (pH 7.4, 37 °C) for 30 min. The dried heart was removed of the right atria and the right ventricle, and frozen with 3% sodium carboxymethylcellulose (CMC) in a refrigerator. The block was cut into slices of 125 μm in width with a microtome for autoradiography, and fixed on an adhesive tape. The uninfarcted myocardium was stained with brick red due to the presence of the dehydrogenase enzymes, whereas infarcted regions remained unstained due to the lack of the enzymes. The boundaries of the three parts, normal (blue), area at risk (brick red) and infarcted (pale white), were carefully traced on a clear transparent sheet and the area of each region was measured by planimetry.

4. Drugs Used — rtPA (GMK-527, Genentech Inc. Lot #H90791B) containing excipient was diluted with saline and used for the study. Dogs in the 360 min-ischemia group received the excipient only.

5. Statistics — All data are expressed as mean ± standard errors except for those in Fig. 2, in which the standard errors are omitted for the purpose of clarity. Mean arterial blood pressure and heart rate were analyzed by paired t-
test. The infarct size was analyzed by Dunnett test after analysis of variance (ANOVA). The changes in ST-segment were compared by Scheffe test after Kruskal–Wallis analysis. p values less than 0.05 were considered statistically significant.

### Results

In the 360 min-ischemia group, spontaneous thrombolysis was not observed throughout the experiment. In contrast, coronary thrombolysis was achieved in all the dogs that received rtPA (10 μg/kg/min, for 30 min) (60 min-ischemia group, n = 5; 30 min-ischemia group, n = 5) except in one in the 60 min-ischemia group. This dog received intracoronary additional rtPA and achieved thrombolysis. Total ischemic periods in the 60 min-ischemia group and the 30 min-ischemia group were 90.0 min (75—120 min) and 45.0 min (35—60 min), respectively.

As shown in Table I, mean arterial blood pressure in the 60 min-ischemia group significantly decreased at 30 min and 60 min after reperfusion, but it gradually recovered to the prethrombus value. Heart rate in the 360 min-ischemia group slightly increased during the experiment. In general, changes in mean arterial blood pressure and heart rate were not prominent in all 3 groups throughout the experiment.

The effects of reperfusion by rtPA on infarct size are shown in Fig. 1. All groups exhibited an area at risk representing 38—45% of the total volume of the left ventricle. These values were not significantly different from each other, which indicates that the extent of ischemic insult is uniform in all the three groups of dogs. The area of necrotic tissue as percent of the area at risk (I/AR) amounted to 46.4 ± 3.3% and 30.4 ± 3.8% in the 60 min-ischemia and in the 30 min-ischemia group, respectively. These values are significantly smaller than that in the 360 min-ischemia (vehicle-treated) group (71.8 ± 4.1%).

![Fig. 1. The Effect of RtPA-Induced Reperfusion on Myocardial Infarct Size](image-url)
Fig. 2. The Effect of RtPA-Induced Reperfusion on Changes in ST-Segment in ECG (Lead II)

Each point represents the mean of 5 experiments except for four points, at which the mean of 4 experiments (4) was shown. Error bars are omitted for the purpose of clarity. a) p < 0.01, b) p < 0.05; significantly different from corresponding value of the 360 min-ischemia group.—○—, 360 min-ischemia (n = 5); —●—, 60 min-ischemia (n = 5); —△—, 30 min-ischemia (n = 5).

The same tendency was observed as for I/LV.

The changes in ST-segment in Lead II ECG are shown in Fig. 2. RtPA-induced reperfusion markedly ameliorated the ischemic changes in ECG in the 60 min- and 30 min-ischemia groups. The ameliorative effects were striking especially in the latter half of the experimental period.

Discussion

Occlusion of coronary artery causes ischemia to the perfused bed of this artery and, finally, makes these ischemic cells die. However, progression of cell death does not spread instantaneously, but gradually spreads from the subendocardial to subepicardial myocardium (i.e., wavefront phenomenon). Thus, some of the jeopardized myocardium can be salvaged if reperfused within several hours after occlusion. In experimental studies using dogs, Flammeng et al. showed that the duration of LAD occlusion was closely related to the infarct size. The calculated infarct sizes by the regression curve expressed as a percentage of the area at risk after 60 and 120 min of occlusion are 28% and 64%, respectively. Permanent occlusion produces an infarct that amounts to 80% of the ischemic bed at risk. On the other hand, Lavallee et al. reported that significant recovery of systolic shortening and velocity of shortening gradually occurred over the 4-week period following reperfusion when reperfused at 60 min after occlusion. However, the recovery was less pronounced if occlusion lasted for 120 min and significant recovery of these functional changes was little observed in dogs subjected to 180 min of occlusion even at 4 weeks after reperfusion.

Based on the above theory, we assumed that the occlusion period should be less than 120 min. In fact, salvageable ischemic myocardium is only 20% ([(80 – 64)/80 = 16/80] if the ischemic period lasts for 120 min, while 65% ([(80 – 28)/80 = 52/80] of the ischemic myocardium can be salvaged if the duration of ischemia is only 60 min. Furthermore, recovery of cardiac function might be expected when the reperfusion is achieved in 60 min. Therefore, we established two groups of different duration of ischemia, 30 and 60 min, in order that the effect of reperfusion will be certainly detectable.

In the present study, the infarct size was significantly reduced by early thrombolysis by rtPA. This result is in agreement with those obtained in the experiments using baboons (reperfused at 38—108 min after occlusion) and using dogs (reperfused at 90—150 min after occlusion). The infarct size in the 30 min-ischemia group was smaller than that in the 60 min-ischemia group. This result agrees with the theory of wavefront phenomenon. These two results definitely demonstrate that early thrombolysis is beneficial to the salvage of ischemic myocardium. It appears from these results that damage to the reperfused myocardium caused by intramyocardial hemorrhage or oxygen-derived free radicals can be overcome by early thrombolysis.

Kopla et al. reported that the reduction in infarct size was also observed even in dogs whose thrombi streptokinase failed to lyse. From that observation, they suggested that streptokinase may reduce the infarct size by a mechanism that is independent of its ability to lyse thrombi. If rtPA also has such action, the beneficial effect of rtPA-induced thrombolysis in the present study may be overestimated.

In most cases, recanalization of coronary ar-
Thrombolysis on Infarct Size

...by thrombolytic agents cannot bring about recovery of regional or global left ventricular function in several hours after reperfusion (i.e., stunned myocardium). The reason has been attributed to the abnormality of left ventricular segmental function, especially to the elevation of end-diastolic wall thickness presumably due to cell swelling caused by rapid increase of blood flow. However, this temporary attenuated left ventricular function usually recovers in several weeks after reperfusion. At 4 weeks after 60 min of occlusion followed by reperfusion, regional left ventricular function recovered almost to the same level as the pre-experimental value. From these results, we might expect that the reduction in infarct size will eventually lead to the improvement of left ventricular function in the chronic phase of post-infarction. In fact, in clinical situations, the greater the extent of reduction of infarct size, the greater the improvement of left ventricular ejection fraction.

In conclusion, using a canine model of thrombin-induced coronary thrombosis, we have demonstrated that the thrombolytic reperfusion by rtPA within 120 min after the onset of myocardial ischemia is beneficial to the salvage of jeopardized myocardium. The shorter the duration of ischemia, the less the extension of infarct. The present results support the notion that coronary recanalization should be achieved as early as possible.

References