

RECOMBINANT SINGLE-CHAIN UROKINASE-TYPE PLASMINOGEN  
ACTIVATOR (rscu-PA) INDUCES THROMBOLYSIS AND SYSTEMIC  
FIBRINOLYSIS IN A CANINE MODEL OF CORONARY ARTERY THROMBOSIS

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**Abstract:** The thrombolytic efficacy of recombinant single-chain urokinase-type plasminogen activator (rscu-PA) was studied in an open-chest canine model of coronary artery thrombosis. Dogs (n=16) were anesthetized, a left thoracotomy performed, and a two cm segment of the left circumflex coronary artery was isolated and instrumented with an electromagnetic flow probe, an intracoronary stimulation electrode, and an adjustable mechanical occluder. Anodal direct current (100  $\mu$ A) was applied to the stimulation electrode until thrombosis occurred (n=14). After 30 min of thrombotic occlusion, rscu-PA was administered intravenously. Dogs were sacrificed either 6 h after thrombolysis or 6.5 h after initiation of rscu-PA when thrombolysis did not occur. In group A (30-50  $\mu$ g/kg bolus rscu-PA + 20-40  $\mu$ g/kg/min infusion rscu-PA for 30 min, n=5) thrombolysis occurred in one case (20%) and this artery reoccluded. In group B (250  $\mu$ g/kg bolus rscu-PA + 25  $\mu$ g/kg/min infusion rscu-PA for 30 min, n=6) all reperfused and only one reoccluded (16.6%). In group C (200  $\mu$ g/kg bolus rscu-PA + 100  $\mu$ g/kg/min rscu-PA infusion for 30 min, n=2) both reperfused and neither reoccluded. Infarct size, determined as a percentage of left ventricle, was smaller when thrombolysis was followed by persistent reperfusion (n=7), than when reperfusion did not occur (n=4):  $16.9 \pm 3.7\%$  vs  $31.3 \pm 2.2\%$ , respectively (mean  $\pm$  SEM,  $p < 0.02$ ). If thrombolysis was followed by reocclusion, infarct size was  $27.0 \pm 10.0\%$ . In this study thrombolysis occurred when changes in prothrombin time, partial thromboplastin time, fibrinogen and fibrin split products were suggestive of systemic fibrinogenolysis. In conclusion, effective thrombolysis with rscu-PA appears to limit infarct size and to be accompanied by evidence of systemic fibrinolysis.

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Keywords: Pro-urokinase, rscu-PA, thrombolysis, myocardial infarction, fibrinogenolysis

## **Introduction**

Thrombolytic therapy in the early stage of evolving myocardial infarction has been shown to be beneficial clinically (1-4). However, defining the optimal dose regimen which would provide rapid thrombolysis and prevent reocclusion without significant bleeding complications has been difficult (5). The clot specific fibrinolytic agents, tissue-type plasminogen activator (t-PA) and recombinant single-chain urokinase-type plasminogen activator (rscu-PA), were expected to be more effective and safer thrombolytic agents.

The mechanism of action for clot-specific thrombolysis is different for t-PA and rscu-PA. Specific plasma inhibitors of t-PA may limit its action in the absence of fibrin (6). The clot specificity of t-PA is due to its affinity for plasminogen in the presence of fibrin (7). The fibrin selective clot lysis of scu-PA is due to activation of plasminogen bound to fibrin in the lys-plasminogen-like conformation, while the usual circulating glu-plasminogen form is spared (8). Plasma inhibitors of scu-PA have not been demonstrated. However, different forms of single-chain urokinase are to be expected to have different half-lives and enzyme activities (9).

In experimental studies, scu-PA and rscu-PA were found to be effective thrombolytic agents which did not induce systemic fibrinolysis unless a dose was used which exceeded the relative threshold of specificity (10-16). Species variability in fibrinolytic responses to rscu-PA has been reported (15), as has fibrinogenolysis with high doses of rscu-PA (15,16). When used in a canine model of coronary artery thrombosis, both t-PA and rscu-PA were effective without inducing systemic fibrinolysis when the thrombus was generated with a copper coil (11,12,17). However in humans and in the canine model of coronary artery thrombosis that was used in this study, rscu-PA and rt-PA caused systemic fibrinolysis when administered in a sufficient dose to achieve effective thrombolysis (18-21). Whether or not these laboratory findings predict clinically significant bleeding complications has yet to be established.

We investigated rscu-PA in a well-defined canine model of coronary artery thrombosis (22) to determine its (a) thrombolytic efficacy, (b) ability to maintain vessel patency after initial thrombolysis, (d) systemic fibrinolytic effects, and (e) influence on infarct size after thrombosis/thrombolysis and reperfusion myocardial injury.

## **Methods**

**Surgical Preparation:** Sixteen male mongrel dogs (14-21 kg) were anesthetized with pentobarbital sodium (30 mg/kg i.v.), intubated and ventilated with room air at a tidal volume of 30 ml/kg and a frequency of 12 breaths/min (Harvard respirator). The left carotid artery and internal jugular vein were exposed and catheters were inserted for monitoring arterial pressure (Statham P23DC pressure transducer) and infusion of drugs, respectively. A left thoracotomy was performed in the fifth intercostal space and the heart was suspended in a pericardial cradle. A two cm section of the left circumflex coronary artery was isolated proximal to the first marginal branch and instrumented proximal to distal as follows: electromagnetic flow probe (Carolina Medical Electronics Model 501), stimulation electrode and a teflon screw-occluder. The stimulation electrode consisted of a 25-gauge hypodermic needle tip attached to a 30-gauge Teflon-insulated silver-coated copper wire (Figure 1). The occluder was adjusted to decrease the reactive hyperemic flow by 50-70% without affecting mean resting coronary blood flow (23) (Figure 2). The small intervening coronary branches over the 1-2 cm segment were ligated. Continuous recordings of blood pressure, the standard lead II electrocardiogram, mean and phasic left circumflex coronary artery blood flow were recorded on a Grass Model 7 polygraph. Zero flow and hyperemic flows were determined by occluding the circumflex coronary artery distal to the flow probe for 10 sec. The flow probe was calibrated using heparinized whole blood. Dogs were allowed to stabilize after the surgical procedure for 30 min before the electrical stimulation was started. If thrombosis occurred, 30 mg of lidocaine hydrochloride was given intravenously. Six hours after thrombolysis or 6.5 h after thrombotic occlusion (if no thrombolysis occurred), the dogs were euthanized and the hearts removed quickly for postmortem quantification of infarct size and determination of thrombus mass (mg).

**Postmortem quantification of infarct size:** In order to determine the thrombus mass, it was necessary to dissect and remove a portion of the coronary artery. Therefore quantification of the area at risk for infarction with the dual staining technique was not possible (24). Rather, the heart was cut into six equal sections, 1.0-1.5 cm thick, perpendicular to the apex-base axis and immersed in 1.5% triphenyltetrazolium hydrochloride in 20 mM potassium phosphate buffer (pH 7.4, 38°C) for 5 min. The presence of dehydrogenase enzymes in viable myocardial regions leads to reduction of tetrazolium salt and the development of a red formazan precipitate in the heart tissue. Infarcted tissue did not reduce tetrazolium salt and remained pale tan in color (25, 26). The sections were trimmed of right ventricular, valvular, and fatty tissue and weighed. Infarct size was determined using computer-assisted planimetry. Infarct size was expressed as percentage of the total left ventricle.

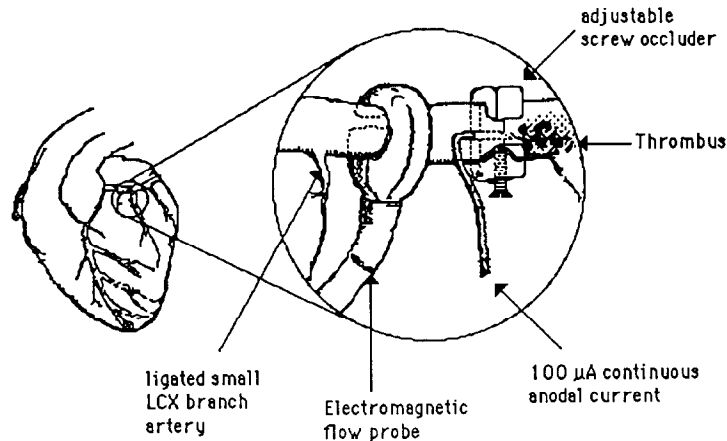
**Coagulation Studies:** The prothrombin time (PT) was determined with thromboplastin C [dried rabbit brain thromboplastin with calcium (Becton, Dickinson and Co., Rutherford, NJ 07070)] The maximum time measured was 60 sec. Activated partial thromboplastin time (PTT) was determined using calcium chloride (0.02 M) and activated cephaloplastin reagent [liquid rabbit brain cephalin with plasma activator (Becton, Dickinson and Co., Rutherford, NJ 07070)]. The maximum time measured was 150 sec. Fibrinogen levels were determined with thrombin reagent [lyophilized bovine thrombin, 100 NIH units/ml (Becton, Dickinson and CO., Rutherford, NJ 07070)] and Owren's veronal buffer M sodium barbital in 0.125 M NaCl, pH 7.35). The minimum amount measurable was 15 mg/dl. Control plasma for the PT, PTT, and fibrinogen assays was a lyophilized preparation of human plasma [Ci-Trol Coagulation Control, Level I (American Dade, Aguada, Puerto Rico 00602)]. Fibrin(ogen) split products (FSP) or degradation products (FDP) were measured with immunological methods observing the agglutination of Latex Anti-Fibrinogen [0.6% suspension of polystyrene latex particles coated with rabbit anti-human fibrinogen in buffer (American Dade, Aguada, Puerto Rico 00602)] and Sorensen's glycine buffer (0.1 M glycine and 0.1 M saline, pH 8.2). Sample tubes for FSP assays contained thrombin (20 NIH units) and soy bean trypsin inhibitor [approx. 3600 NF units (Wellcome Laboratories, Beckenham, U.K.)]. Vacutainer tubes for PT, PTT and fibrinogen contained 0.5 ml of 3.2% buffered sodium citrate (Becton Dickinson, Rutherford, N.J.). Platelet counts were determined using a J.T. Baker MK/4Hc platelet counting system.

**Drugs:** Recombinant single-chain urokinase-type plasminogen activator, a gift from Grünenthal GmbH (Aachen, West Germany, Drs. Flohe and Günzler), was produced from E. coli sources ( $M_r$  47,000) and contained 0.09% double chain urokinase activity. Lidocaine hydrochloride was purchased from Sigma (St.Louis, Mo.).

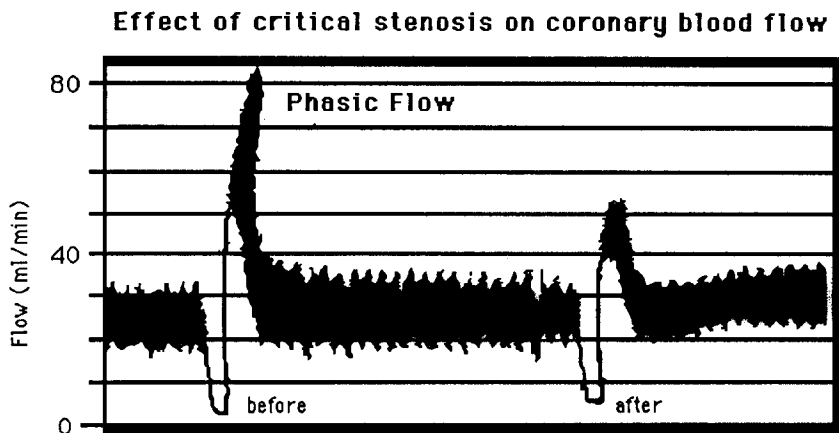
**Statistical Analysis:** The data are expressed as the mean  $\pm$  SEM (standard error of the mean). The groups were compared using one way analysis of variance and Student's t-test. A p value less than 0.05 was considered significant. Changes within groups were established by repeated measures of ANOVA and significance between means was determined using Scheffe's test.

**Experimental Protocol:** After surgical preparation, the dogs were allowed to stabilize and then the electrical stimulation was initiated. If complete occlusive thrombus was not established within 4 h the dogs were excluded from the study. If coronary artery thrombosis occurred, as assessed by zero coronary blood flow and ST segment-elevation in the lead II electrocardiogram, then electrical stimulation was discontinued 30 min after thrombotic occlusion and thrombolytic treatment was initiated. Blood samples for coagulation studies were taken initially and at 5 and 30 min after the bolus was given and 1, 3 and 6 h after the initiation of the treatment. Three rscu-PA treatment groups (varying dose schedules) were studied:

- Group A: 30-50  $\mu$ g/kg bolus + 20-40  $\mu$ g/kg/min infusion over 30 min  
(total dose = 0.64-1.25 mg/kg, n=5)
- Group B: 250  $\mu$ g/kg bolus + 25  $\mu$ g/kg/min infusion over 30 min  
(total dose = 1 mg/kg, n=6)
- Group C: 200  $\mu$ g/kg bolus + 100  $\mu$ g/kg/min infusion over 30 min  
(total dose = 3.2 mg/kg, n=2)



**Figure 1:** Instrumentation of the left circumflex coronary artery.



**Figure 2:** Phasic coronary blood flow before and after adjustment of the critical stenosis. The hyperemic response is reduced approximately by 50%, whereas the mean basal flow is unaffected.

### **Results**

A total of 16 dogs were used in the study. Two dogs were excluded because they did not develop coronary artery thrombosis after 4 h of electrical stimulation. Since postmortem examination of the vessel revealed that the wire had been in place, it was concluded that the lack of thrombus formation was due to initial low platelet counts ( $<50,000/\text{mm}^3$ ). One dog was excluded because the infusion was accidentally started 30 min after the bolus. Interestingly, reperfusion was achieved in this case. Since group C is small ( $n=2$ ), the results of this group will be presented separately. The following paragraphs compare the results of groups A and B.

**Thrombosis, thrombolysis, reocclusion, and thrombus weight:** There was no significant difference between groups A and B with respect to the time required to develop a fully occlusive thrombus ( $75.2 \pm 24.3$  min vs  $98.8 \pm 26.5$  min, respectively). In group A, thrombolysis occurred in 1/5 (20%) and this was followed by reocclusion. In group B, thrombolysis occurred in all 6, and only 1/6 (16.6%) reoccluded. The time necessary to achieve thrombolysis for the one case in group A

was 25 min and for all 6 cases in group B was  $16.3 \pm 4.3$  min. The difference in thrombus weight between the two groups nearly reached significance ( $30.2 \pm 7.0$  mg group A vs  $10.9 \pm 5.7$  mg group B,  $p=0.06$ ). In group A, reocclusion occurred 95 min after thrombolysis ( $n=1$ ); whereas only 1/6 vessels reoccluded in group B and this occurred after 20 min. Reocclusion developed gradually and was not accompanied by oscillations in coronary blood flow as has been described by Schumacher et al (26).

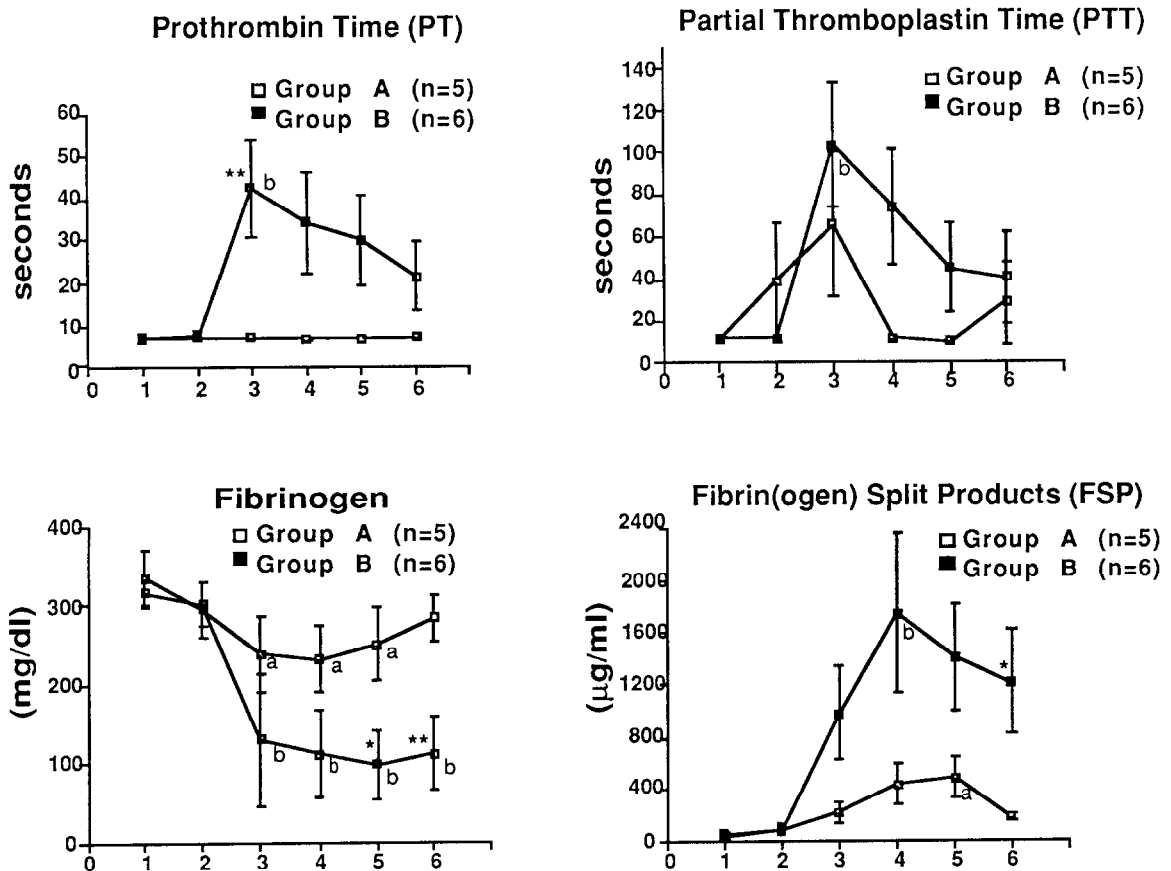
**Hemodynamics and coronary blood flow:** In the beginning of the experiments there was a tendency towards higher mean arterial pressure (MAP) and heart rate (HR) in group B compared to group A. These differences however became less prominent over the course of the experiment and final values were not different. Left circumflex coronary blood flow (CBF) was similar before thrombosis ( $20.5 \pm 2.2$  ml/min group A vs  $27.3 \pm 5.5$  ml/min group B). Neither the administration of a low dose or high dose bolus, nor the 30 min infusion had an effect on MAP, HR or CBF. In group A thrombolysis occurred in one dog, whereas in group B thrombolysis occurred in all [initial reperfusion CBF: 18 ml/min ( $n=1$ ) vs  $11.7 \pm 3.7$  ml/min ( $n=6$ ), respectively]. In the patent vessels of group B, CBF was  $15.4 \pm 3.6$  ml/min ( $n=5$ ) after 6 h of reperfusion, a trend toward increasing CBF over time. By this point in time, the one patent vessel had reoccluded in Group A.

**Coagulation studies:** In preliminary experiments it was established that the soy bean trypsin inhibitor successfully inhibits activation of plasminogen to plasmin while the sample was being processed. This was essential to ensure that the measured fibrin/ogen split or degradation products (FSP or FDP) were not an artifact that occurred in the test tube due to ongoing activation by a tissue-type plasminogen activator (27). When samples were taken 5 min after bolus injection there was no detectable difference in prothrombin time (PT), partial thromboplastin time (PTT), FSP or fibrinogen measured initially or after several hours, thus indicating that plasminogen activation in the test tube was negligible and that the soy bean inhibitor was effective in preventing proteolysis of fibrinogen by plasmin. Compared to normal human plasma, the values in dogs for PT and PTT were 50% shorter, fibrinogen was similar, and FSP were minimally elevated after thoracotomy. The values for PT, PTT, fibrinogen and FSP were similar between the two groups before treatment (baseline values were taken after thoracotomy). Platelet counts were similar for the two groups (data not shown).

The PT for group B ( $42.3 \pm 11.2$ ) was prolonged at 30 min after the onset of treatment compared to group A ( $p<0.02$ ) and there was a significant change over time within group B. Over time there were no changes detected in the PT for group A. The PTT in group B ( $104.1 \pm 29.0$ ) was prolonged at 30 min and this was a significant increase compared to baseline. Fibrinogen was lower in group B than group A 3 h and 6 h after starting therapy ( $p<0.05$  and  $p<0.02$ , respectively). Within both groups there were significant decreases in fibrinogen over time: in group A at 30 min, 1 h and 3 h and in group B from 30 min throughout 6 h. The FSP were increased in group B compared to group A at 6 h ( $p<0.05$ ). Within the groups significant increases for FSP were present over the course of the experiments: in group A at 3 h and in group B at 1 h.

It seems that abnormalities in the coagulation pathway were necessary for thrombolysis to occur. There was a tendency to normalization of the abnormal coagulation parameters towards the end of the experiments. The lack of differences within and between groups and the large SEM with respect to coagulation parameters was an indicator of the variability of the dog's response to fibrinolytic agents. Statistical analysis of the data for PT and PTT may have been influenced by arbitrary upper limits of sensitivity of the assays (60 sec and 150 sec, respectively). It is important to note that none of the dogs exhibited substantial bleeding, even though an open-chest model was used. A summary of the findings in regard to coagulation parameters is given in figure 3.

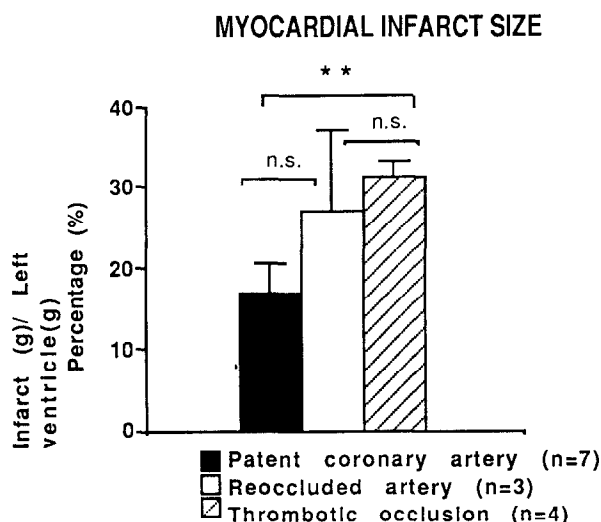
**Infarct size:** Although measurements of infarct sizes, especially when the area at risk is not defined and when collateral blood flow is not measured, have to be noted with caution, postmortem infarct sizes can still be regarded as a reasonable estimate of myocardial tissue injury. Infarct size, expressed as percentage of the left ventricle, was smaller in groups B than group A ( $19.0 \pm 3.4\%$  vs  $34.4 \pm 3.6\%$ , respectively,  $p<0.02$ ). This finding suggested that thrombolysis occurring early in evolving myocardial infarction reduced infarct size. Whether or not there were additional



**Figure 3:** Coagulation profile over the time of the experiment. 1=Baseline, 2=5 min after bolus, 3=30 min after bolus, 4=1 h after bolus, 5=3 h after bolus, 6=final. \*= $p < 0.05$ , \*\*= $p < 0.02$ , a=differences within Group A compared to baseline, b=differences within group B compared to baseline (a+b  $p < 0.05$  with Scheffe's test).

mechanisms for protecting ischemic myocardial cells when a fibrinolytic agents was employed in order to restore coronary blood flow needs further investigation. Infarct sizes from groups A, B, and C were analyzed according to whether thrombolysis occurred, and if so did reocclusion occur, the smallest infarct size was found in the group with initial thrombolysis and persistently patent vessels ( $16.9 \pm 3.7\%$ ,  $n=7$ , Figure 4) and this was significantly smaller than the infarct sizes when thrombolysis never occurred ( $p < 0.02$ ). In the computation of infarct sizes according to patency the dog where the infusion was started late was included.

**Results for Group C:** In the two dogs that received 3.2 mg/kg total dose of rscu-PA, extensive bleeding occurred necessitating significant crystalloid replacement to maintain hemodynamic stability. Changes in the measured coagulation parameters were profound 30 min after initiation of treatment: PT ( $>60 \pm 0$  sec), PTT ( $>150 \pm 0$  sec), fibrinogen ( $<15 \pm 0$  mg/dl) and FSP ( $4608.0 \pm 3584.0$   $\mu$ g/ml). The PT and PTT are dependent upon fibrinogen from the plasma sample, and when it is depleted ( $\leq 15$ mg/dl) as in these studies, all three tests will be abnormal. The increased FSP suggest that the low fibrinogen was induced by systemic fibrinogenolysis. By the end of the experiments, the values showed a tendency towards normalization: PT  $36.5 \pm 23.5$  sec, PTT  $84.4 \pm 65.7$  sec, fibrinogen  $15.5 \pm 0.5$  mg/dl, FSP  $4096 \pm 0$   $\mu$ g/ml. Systemic fibrinogenolysis resulted in a time to induce reperfusion which was short ( $12.6 \pm 3$  min), infarct size which was small ( $12.9 \pm 4\%$ ) and thrombus mass which was small ( $1.3 \pm 1.3$  mg). Since group C was small



**Figure 4:** Myocardial infarct size grouped according to the incidence of thrombolysis with persistently patent vessels, thrombolysis with reocclusion, and thrombolysis due to failure of thrombolytic therapy. ( \*\* =  $p < 0.02$  )

(n=2), any conclusions based on these data must be considered cautiously.

### Discussion

Clinically, effective thrombolytic therapy used early (<4h after onset of symptoms) in acute myocardial infarction has been shown to improve myocardial function and patient survival (1-4). Streptokinase was the first thrombolytic agent used and it is the only agent where data exist to support the hypothesis that long-term mortality is reduced (3). Because of several problems with streptokinase, i.e. bleeding, antigenicity and rethrombosis, there is now a wide-spread interest in the second-generation thrombolytic agents which are more clot specific. Recombinant human tissue-type plasminogen activator (rt-PA) has been used for coronary thrombolysis in acute myocardial infarction and the results from several multicenter studies are available (4,28-30). Effective thrombolysis in humans according to those studies is accompanied by systemic fibrinolysis. In an animal study carried out in this laboratory, 1 mg/kg bolus tPA + 1 mg/kg tPA infused over 3 hours (5.5  $\mu\text{g}/\text{kg}/\text{min}$ ) was an effective coronary thrombolytic agent in 78% of cases (7/9 dogs) and systemic fibrinolysis also was noted (18).

Systemic fibrinolysis was not detected in previously reported preliminary experiments with scu-PA. However, there are now data available suggesting, that in humans the dose necessary to induce coronary artery thrombolysis also causes systemic fibrinogenolysis (20,21). Again, the dose found to be effective (31), was similar to the dose that was effective in the study reported herein. Systemic fibrinogenolysis was detected in our study and there may be several reasons for this: (1) effective doses are high enough to exceed the relative threshold of specificity of scu-PA and (2) inactivation of plasmin by alpha-2 antiplasmin was exceeded by the excessive formation of plasmin in the model.

One of the striking results of this study is the finding that the same total dose administered in different regimens does produce remarkably different efficacy. Based on a one compartment model in dogs and an estimated  $t_{1/2}$  of 7.5 min for rscu-PA both regimens (group A and B) reach plasma concentrations of more than 100 nM. According to a report from Collen et al (32) in vitro lysis of a human plasma clot was achieved within 1h when scu-PA was present at a concentration of 32 nM. Neglecting the interspecies differences one would assume that thrombolysis should occur in both groups A and B described in this study. Based upon the results of this study one might

conclude the following: (a) there is a dose dependency of the velocity of the activation of plasminogen by rscu-PA and (b) the sigmoid dose response that was found by Collen et al (32) may be valid for actual plasma concentrations in an in vivo system. It is possible that the fibrin present in the thoracic wound in this model binds part of the administered fibrinolytic agent which results in a higher total dose needed to lyse the coronary artery thrombus.

At this point it is important to discuss the differences between the canine model used in this study and other studies. Collen et al (11,12) describe effective thrombolysis being achieved without systemic fibrinogenolysis at a dose that was approximately three times smaller than the dose used in our study (0.3mg/kg vs 1mg/kg, respectively). Thrombosis induced with an intracoronary copper coil in the model used by the Leuven group and thrombosis induced via electrical stimulation of the coronary artery intima as in this study generate different types of thrombi with respect to the composition of the clot. Romson et al. studied the electrically induced thrombus and found that it resembles the platelet-rich thrombi found in postmortem studies of humans with coronary artery disease (22). Differences between the experimental models may account for the different findings with rt-PA. Lysis in our canine model was accompanied by systemic fibrinogenolysis (18) and the dose needed for thrombolysis was approximately three times higher than that used by the Leuven group (17). In a recent study, histology of the thrombi revealed the crucial role of the platelets as well as the changing composition of the thrombi over a short period of time which led to significant reduction of effective thrombolysis with urokinase (33). It is of interest that the doses necessary to induce thrombolysis in our model are similar to those proven to be effective in humans for both rt-PA and rscu-PA and that systemic fibrinolysis is present with both recombinant agents. Although the drugs were not studied simultaneously, it can be suggested that rt-PA and rscu-PA are both effective in this model of coronary artery thrombosis/thrombolysis and that thrombolysis is usually achieved in the presence of systemic fibrinolysis. In these studies, the laboratory evidence of systemic fibrinolysis did not correlate with overt bleeding in the open-chest model and severe blood loss only occurred at a total dose three times higher than necessary to achieve effective reperfusion with rscu-PA.

The mechanism of reocclusion after thrombolytic therapy is not fully understood. It is conceivable that platelets play a role in this phenomenon. In the protocol employed in this study, coronary blood flow is monitored continuously, thus the pattern of coronary blood flow leading to reocclusion can be observed. Oscillations in coronary blood flow have preceded reocclusion in studies using this model with other thrombolytic agents (18, 26). The observed oscillations are similar to those seen when a circumferential coronary artery stenosis is present and occlusion occurs, and these oscillations can be abolished with ketanserine (34). With electrically induced thrombosis, prolonged vessel patency after streptokinase improved with prostacyclin and heparin (35), and initial thrombosis could be prevented with ibuprofen (23) or thromboxane synthetase inhibition (36). Streptokinase and t-PA have been reported to cause hyperaggregation of platelets in rabbits (37). Although platelet activation has not been studied in dogs in this model, it could be contributing to the oscillations or cyclic flow variations in coronary blood flow which precede reocclusion. These findings indicate that the mechanisms of thrombosis and thrombolysis involve a complex interaction involving the vascular endothelium, circulating cellular elements and plasma clotting factors and their inhibitors. To our knowledge rscu-PA has not been reported to cause platelet hyperaggregability. The presence of platelets in a thrombus has, in fact, been reported to augment rscu-PA induced lysis (38). Since in this study there was a low rate of reocclusion and the flow pattern preceding reocclusion was different from studies with streptokinase and t-PA, it will be important to investigate the rheological and platelet activating properties of rscu-PA and other proposed thrombolytic agents.

**Conclusions:** Recombinant single-chain urokinase plasminogen activator is an effective thrombolytic agent in this model of coronary thrombosis at doses comparable to those necessary for thrombolysis in humans. The high initial bolus (1/4 of the total dose) is impressive in its ability to promote early thrombolysis. When a small initial bolus is used with a similar total dose, it is not effective. Further investigation is needed to resolve the following questions: 1) Should a loading dose which is 25% of the total dose be used as the clinical therapeutic regimen? 2) Should different methods of measuring coagulation factors be developed in order to distinguish between



lytic states that endanger patients and those that merely indicate effective thrombolysis?

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