Requirement of Heparin for Arterial and Venous Thrombolysis With Recombinant Tissue-Type Plasminogen Activator

By Hans J. Rapold, Hua R. Lu, Zaomin Wu, Herman Nijss, and Désiré Collen

The effect of concomitant intravenous (IV) heparin (200 U/kg bolus, followed by 100 U/kg/h) on the efficacy of arterial and venous thrombolysis with IV recombinant tissue-type plasminogen activator (rt-PA; 0.5 mg/kg over 1 hour) was investigated in a combined femoral arterial and venous thrombosis model in the dog. The arterial model consisted of a high-grade stenosis, endothelial damage, and a thrombotic occlusion, and the venous model consisted of a 120% fibrin-labeled blood clot. After a dose-finding pilot study in four dogs, a randomized, prospective, and blind study was performed in 20 animals pretreated with 2.8 mg/kg IV acetylsalicylic acid (ASA). The combination of rt-PA and heparin (group I, n = 10) induced early (<30 minutes) arterial reperfusion in seven dogs, late (>30 minutes) reflow in two dogs, and persistent occlusion in one dog. rt-PA alone (group II, n = 10) was associated with early reperfusion in one dog, late reflow in three dogs, and persistent occlusion in six dogs (P = .018). Reocclusion occurred in five of nine reperfused dogs of group I and in one of four reperfused dogs of group II (P = not significant). Venous clot lysis amounted to 81% ± 4% (mean ± SEM) for group I and to 49% ± 7% for group II (P < .001). Template bleeding times increased moderately, but significantly, in group I (from 2.2 ± 0.2 minutes at baseline to 7.0 ± 1.4 minutes at 30 minutes, P = .006), but only marginally in group II (from 2.2 ± 0.2 minutes to 3.6 ± 0.7 minutes, P = .09). No systemic fibrinogen depletion was observed. Thus, the concomitant use of heparin with rt-PA accelerates arterial reperfusion and enhances venous thrombolysis in dogs pretreated with ASA. These results, obtained in a randomized prospective study design, add to a growing body of experimental and clinical evidence, indicating that thrombolytic therapy with rt-PA requires concomitant adjunctive IV heparin for optimal efficacy, even in the face of treatment with ASA.

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EARLY THROMBOLYTIC therapy has become the treatment of choice for acute myocardial infarction and is under investigation for other conditions of arterial or venous thrombosis, such as stroke, pulmonary embolism, and deep venous thrombosis. The requirement of adjunctive measures to enhance and accelerate reperfusion and to prevent reocclusion of successfully reperfused vessels is still a matter of debate. Anticoagulation and, in particular, the importance of concomitant heparin infusion for thrombolytic treatment with recombinant tissue-type plasminogen activator (rt-PA), are major concerns for the interpretation of the results of the recently reported GISSI-2 (Gruppo Italiano per lo Studio della Streptochinasi nell’Infarto Miocardico) trial and the International t-PA/Streptokinase Mortality Trial. Experimental animal studies have shown a marked acceleration of rt-PA–induced arterial thrombolysis by pretreatment with heparin or with a defibrinogenating agent. Administration of either rt-PA or streptokinase to patients with acute myocardial infarction generates thrombin activity that can be controlled by heparin administration. Thus, therapeutic anticoagulation, initiated concomitantly with the thrombolytic agent, might prevent ongoing or enhanced fibrin formation. The effect of anticoagulation on the efficacy of thrombolysis could be more significant with fibrin-specific agents, such as rt-PA, that do not cause systemic fibrinogen depletion, than with non–fibrin-specific agents, such as streptokinase. Furthermore, the dissolution of venous blood clots may react differently to anticoagulation than arterial thrombolysis.

The purpose of the present study was to evaluate the effect of heparin on arterial and venous thrombolysis with rt-PA in an animal model of simultaneous arterial and venous thrombosis, using a blind randomized study design.

MATERIALS AND METHODS

Femoral arterial thrombosis model. The animal study conformed to the guiding principles of the American Physiological Society. Adult shepherd dogs weighing 18 to 25 kg were sedated with 0.25 mg/kg fluanisone, anesthetized with 15 mg/kg sodium pentobarbital initially, intubated, and artificially ventilated. Catheters were placed in the left jugular and brachial veins for infusion of agents. The left carotid artery was used for monitoring blood pressure and for withdrawal of blood samples. The right femoral artery was exposed in the inguinal region, and a 3 FG catheter (Portex, Hythe, England) was inserted into a side branch. Other side branches, when present, were ligated. An electromagnetic flow probe (Medelad, Deurne, Belgium) was placed around the distal region of the arterial segment for continuous blood flow monitoring. A 3-mm wide plastic wire tie was progressively constricted around the artery proximally to the flow probe, until the blood flow was reduced to 39% ± 1% of baseline. This constriction corresponds to a greater than 90% reduction in luminal diameter, as estimated by angiography, performed in the first 10 dogs studied. The exposed arterial segment was traumatized proximally to the constriction by three consecutive external compressions with blunt forceps for 5 seconds to promote thrombus adherence to the endothelium. Then an arterial segment was isolated by placing a distal vessel clamp between the constricting plastic tie and the electromagnetic flow probe, and a second clamp at a distance of 2 cm proximally to the first one. Thrombin, 0.1 mL (10 U), was injected into the emptied femoral artery segment through the side-branch catheter, followed by 0.3 to 0.4 mL of blood. The thrombus was allowed to age for 30 minutes before the clamps were released. An angiography was performed in the first 10 dogs to confirm initial total occlusion and subsequent reperfusion, as demonstrated by the electromagnetic flowmeter. The results of
vessel patency, determined by angiographic and electromagnetic flow measurement, were totally concordant. Therefore, angiography was no longer performed in subsequent experiments. After confirmation of complete occlusion of the femoral artery, the vessel clamps used to simultaneously produce a venous blood clot on the contralateral side (see below) were removed, and the infusion protocol was started. Status and extent of femoral artery perfusion were continuously monitored during the following 2 hours.

**Femoral venous thrombosis model.** A blood clot was produced in the left femoral vein, essentially as previously described. Briefly, the femoral vein was exposed in the inguinal region and all side branches ligated, except for a predominant musculocutaneous branch, which was cannulated. After introduction of a woolen thread in the lumen to prevent embolization of the clot, a 4-cm segment of the vein was isolated between two vessel clamps and emptied via the side-branch catheter. The segment was filled with 0.1 mL (10 U) of thrombin solution, followed by a mixture of 0.8 to 1.0 mL of fresh blood and a trace amount of 125I-labeled human fibrinogen (approximately 106 cpn). The timing of the procedure was adjusted to allow a simultaneous aging of the left femoral vein clot and the right femoral artery clot for 30 minutes. The venous vessel clamps were released after confirmation of a totally occluded contralateral femoral artery, and the infusion protocol was started. The radioisotope content of the venous clot was calculated by subtracting, from the original amount of 125I injected into the isolated vein segment, the radioisotope that was adsorbed on cotton swabs placed around the vein segment, and the radioiodine that was washed out from the thrombus into the bloodstream, determined 1 minute after removal of the clamps. At the end of the experiment (1 hour after the end of infusion), the thrombosed segment of the femoral vein was ligated at both ends, removed, and its radioisotope content measured. The degree of clot lysis (CL) was determined as the residual radioactivity in the vein segment and expressed in percent of the radioactivity originally incorporated in the clot. The degree of activator induced lysis (AIL) was calculated by correcting the percent CL for the value of background lysis (BL), determined to be 18% ± 8% in three dogs administered excipient infusion, so that $\text{AIL} = (\text{CL} - \text{BL})/(100 - \text{BL})$. An isotope recovery balance was made by comparing the sum of the total blood radioactivity at the end of the experiment (multiplied by three for extravascular distribution) and the radioactivity in the recovered thrombus with that originally present in the clot.

**Infusion protocols.** The study was prospectively designed to be randomized and blind. All animals received an intravenous (IV) bolus injection of 5 mg/kg l-lysine-acetyl-salicylate (containing 2.8 mg/kg active acetyl salicylic acid, ASA) immediately before the start of the infusion. Heparin was administered as an IV bolus of 200 U/kg, followed by a continuous infusion of 100 U/kg/h for 2 hours. The rt-PA dose was determined in the following manner. A pilot study with 1 mg/kg rt-PA was performed in four dogs, and showed that venous lysis, both in the presence or absence of heparin, was nearly complete (93% ± 4%). Therefore, the rt-PA dose was reduced to 0.5 mg/kg, given as a bolus of 0.05 mg/kg (10%), followed by an IV infusion of 0.45 mg/kg over 1 hour. Twenty dogs were randomly assigned to two treatment groups: (I) rt-PA plus heparin, and (II) rt-PA plus placebo. The randomization sequence was determined with a computerized random number generator. The experiments were performed in two blocks of 10 dogs (five with heparin and five with placebo infusion), allowing for a prospectively declared interim analysis. The animals were observed for 2 hours after the start of the infusion protocol, with continuous monitoring of femoral arterial blood flow distal to the occlusion, arterial blood pressure, and electrocardiogram. No cut-off point between early and late reperfusion was prespecified, but an analysis every 15 minutes compared the results with angiographical data of previous studies. rt-PA (Actilyse R) was obtained from Boehringer-Ingeheim (Ingelheim, Germany), heparin (Heparine R) was from Novo (Bagsvaerd, Denmark), and bovine thrombin (Topostasin R) was from Roche (Basel, Switzerland).

**Hemostasis analyses.** Template bleeding times were measured before the start of the infusion, 30 minutes later, and 30 minutes after the end of rt-PA infusion from standard incisions of 1-cm length made with a surgical blade on the volar surface of the dog’s foreleg. All bleeding times were measured by the same investigator (H.N.). Blood samples were collected on citrate before and at 30, 55, 90, 120, and 150 minutes after the onset of infusion. Samples were cooled on ice and centrifuged for immediate analysis of fibrinogen and activated partial thromboplastin time (APTT).

**Statistical analysis.** The results are expressed as mean ± SEM. Groups were compared using a Fisher’s exact test for discrete variables and unpaired or paired t-tests, as appropriate, for continuous variables.

**RESULTS**

**Femoral artery recanalization and reocclusion.** The results of arterial thrombolysis, obtained by electromagnetic flow measurement, are summarized in Table 1. Baseline femoral arterial blood flow was reduced by the artificial stenosis to a similar degree in both treatment groups (41% ± 2% and 37% ± 2%, respectively; $P = $ not significant). An IV dose of 0.5 mg/kg rt-PA, combined with ASA and heparin (group I), induced reperfusion of the occluded femoral artery in 9 of 10 dogs, while rt-PA and ASA without heparin (group II) resulted in reflow in 4 of 10 dogs ($P = 0.07$). Reperfusion was early (within 30 minutes; at 6, 7, 14, 16, 25, 26, and 28 minutes, respectively) in seven dogs and late (after >30 minutes; at 47 and 115 minutes) in two dogs of group I, as compared with early (at 18 minutes) in one and late (at 36, 50, and 65 minutes, respectively) in three dogs of group II. Analysis of the results grouped as early reperfusion, late reperfusion, and persistent occlusion showed a significant difference between the treatment groups ($P = 0.018$). Recoclusion occurred in five of nine reperfused dogs of group I and in one of four reperfused dogs of group II ($P = 0.66$). There was no significant difference in the extent of reperfusion, as shown in Fig 1, with a reflow of less than 50% of the original flow through the stenosis in one dog of each group, and a reflow of greater than 50% in five dogs of group I and in three dogs of group II at the end of the experiment. Reflow did not exceed 50% of the original flow through the stenosis in two of six dogs with subsequent recoclusion.

**Femoral venous thrombolysis.** Treatment with rt-PA, ASA, and heparin (group I) resulted in 81% ± 4% venous clot lysis, as compared with 49% ± 7% with rt-PA and ASA alone (group II), $P < 0.01$. The difference remained highly significant after correction of background lysis (see Materials and Methods): the AIL was 77% ± 5% for group I and 38% ± 8% for group II ($P < 0.001$).

**Hemostasis analyses.** The results of hemostasis analyses are summarized in Table 1. A dose of 0.5 mg/kg rt-PA did
not cause fibrinogen consumption, whether heparin was added to the treatment or not. A residual fibrinogen plasma level of less than 50% of baseline was observed in one animal only (group II). All control values of APTT in group I were at least six times the baseline value, reflecting extensive thrombin inhibition. Template bleeding times increased significantly with rt-PA, ASA, and heparin (group I) from 2.2 ± 0.2 minutes to 7.0 ± 1.4 minutes (P = .006), but only marginally with rt-PA and ASA (2.2 ± 0.2 minutes to 3.6 ± 0.7 minutes, P = .09). Bleeding times were identical in groups I and II at baseline, significantly different at 30 minutes (7.0 ± 1.4 minutes v 3.6 ± 0.7 minutes, P = .04), but no longer at 120 minutes (6.1 ± 2.4 minutes v 3.9 ± 0.8 minutes, P = .4).

**Table 1. Results of Arterial and Venous Thrombolysis and of Hemostasis Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Group I Heparin (n = 10)</th>
<th>Group II No Heparin (n = 10)</th>
<th>Level of Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow through stenosis (% of baseline)</td>
<td>41 ± 2</td>
<td>37 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Arterial reperfusion (n):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 30 min</td>
<td>7</td>
<td>1</td>
<td>.018</td>
</tr>
<tr>
<td>After 30 min</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Venous lysis (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clot lysis (CL)</td>
<td>81 ± 4</td>
<td>49 ± 7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Activator-induced lysis (AIL)</td>
<td>77 ± 5</td>
<td>38 ± 8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Template bleeding times (min):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>At 30 min</td>
<td>7.0 ± 1.4</td>
<td>3.6 ± 0.7</td>
<td>.04</td>
</tr>
<tr>
<td>At 120 min</td>
<td>6.1 ± 2.4</td>
<td>3.9 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrinogen levels (g/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.4 ± 0.3</td>
<td>1.5 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>At 55 min</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>At 150 min</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>APTT (s):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>21 ± 1</td>
<td>20 ± 1</td>
<td></td>
</tr>
<tr>
<td>At 30 min</td>
<td>177 ± 3</td>
<td>24 ± 1</td>
<td></td>
</tr>
<tr>
<td>At 55 min</td>
<td>180 ± 0</td>
<td>29 ± 2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>At 150 min</td>
<td>178 ± 2</td>
<td>24 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

Data represent mean ± SEM. NS, not significant (P > .05).

**DISCUSSION**

The aim of the present study was to investigate the effect of adjunctive use of heparin on the efficacy of arterial and venous thrombolysis by rt-PA in the setting of pretreatment with ASA. A model of combined arterial and venous thrombolysis was developed in the dog, because this species has been used extensively for the investigation of thrombolytic strategies and because its reactivity to rt-PA is well known. The study design was randomized and blind. To maximize the chance of observing a significant effect, the dose of heparin was selected to produce an extensive anticoagulant state in blood. All animals were pretreated with ASA because of the currently generalized use of aspirin in association with thrombolytic therapy in patients with ischemic heart disease. The dose of rt-PA was selected to obtain submaximal lysis in the absence of heparin. Thus, IV doses of 2.8 mg/kg bolus aspirin and 200 U/kg bolus heparin, followed by 100 U/kg/h for 2 hours and 0.5 mg/kg rt-PA, infused over 1 hour, were chosen.

The results of this study are indicative of a significant role of heparin during thrombolytic therapy with rt-PA, notwithstanding pretreatment with ASA. Indeed, concomitant use of heparin with rt-PA enhanced venous thrombolysis and accelerated arterial reperfusion in the dual-thrombolysis model presented here. At a dose of 0.5 mg/kg infused over 1 hour, rt-PA alone produced 49% ± 7% venous lysis, early arterial reperfusion in 1 of 10 dogs, and late reflow in an additional three dogs. The combination of 0.5 mg/kg rt-PA and 200 U/kg bolus heparin (followed by 100 U/kg/h as
infusion) achieved 81% ± 4% venous clot lysis (P < .001), early reperfusion in 7 of 10 dogs, and late reflow in two additional dogs (P = .016). However, reocclusion was not significantly different in the presence (five of nine dogs) or absence (one of four dogs) of heparin.

Our results confirm and extend earlier observations obtained in an animal model with nonocclusive arterial thrombi, which suggested that thrombolysis is enhanced by pretreatment with heparin. Because heparin has no direct fibrinolytic activity, its effect on thrombolysis is likely to be mediated via inhibition of new fibrin deposition into the thrombus during thrombolytic therapy, as was shown previously in rabbits with jugular vein thrombosis and in dogs with radiolabeled fibrinogen. Our data indicate that the accelerating effect of heparin on thrombolysis with rt-PA also occurs in the setting of concomitant use of ASA.

Experimental models with a comparatively high resistance to reperfusion and predisposition to reocclusion allow a conceptual approach to the problem of adjunctive anticoagulation with thrombolytic therapy. The extent to which these models are representative for the effect of adjunctive anticoagulation on the outcome of thrombolytic treatment in humans has to be established in clinical studies. Reports of two randomized trials of heparin anticoagulation in patients with acute myocardial infarction treated with rt-PA, in which patency rates of the infarct-related vessel were determined at 7 to 24 hours and at 48 to 72 hours after treatment, have recently become available. A significantly higher patency rate was observed in patients treated with rt-PA and concomitant heparin than in patients treated with rt-PA alone or with rt-PA and aspirin.

On the other hand, very early (90 minutes) patency rates were not found to differ in the TAMI-3 (Thrombolysis and Angioplasty in Myocardial Infarction) trial, whether heparin was added to rt-PA or not; however, the study design did not allow investigation of the effect of anticoagulation on early reocclusion. Including the results of the latest trial of the European Cooperative Study Group with higher patency rates at 48 to 120 hours in patients treated with rt-PA and heparin, there is now sufficient clinical evidence indicating that rt-PA requires concomitant anticoagulation for an optimal efficacy, even in the presence of adjunctive administration of ASA. No significant increase in bleeding complications was observed in patients randomized to rt-PA and heparin as compared to patients treated with rt-PA alone. Whether this also holds for streptokinase remains an important question in the light of the recent GISSI-2 and international rt-PA/streptokinase mortality studies. The observation that defibrinogenating agents enhance thrombolysis with rt-PA in experimental models to the same extent as heparin suggests that the additional benefit from simultaneous anticoagulation may be less important in association with a thrombolytic drug that induces an intense systemic lytic state. A partial resistance to anticoagulation has been observed in patients with acute myocardial infarction treated with streptokinase.

In conclusion, heparin enhances arterial reperfusion as well as venous clot lysis with rt-PA in the aspirin-treated dog. This observation may be useful for the optimal design of future comparative clinical trials of thrombolytic therapy that include rt-PA.

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Requirement of heparin for arterial and venous thrombolysis with recombinant tissue-type plasminogen activator

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