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

By Hans J. Rapold, Hua R. Lu, Zaomin Wu, Herman Nijs, 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 180°-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). Reclosure 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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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 corresponded 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 cpm). 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 as compared with early (at 18 minutes) in seven dogs and late (after >30 minutes) in 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 (18 minutes) 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 = .018). Reocclusion occurred in five of nine reperfused dogs of group I and in one of four reperfused dogs of group II (P = .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 reocclusion.

**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 = .057). 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 (after 18 minutes) in one and late (after 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 = .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). Thus, femoral patency at the end of the experiment was obtained in six dogs of group I versus four dogs of group II (P = .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 reocclusion.

**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 = .057). 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 (18 minutes) in one and late (after 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 = .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). Thus, femoral patency at the end of the experiment was obtained in six dogs of group I versus four dogs of group II (P = .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 reocclusion.

**Femoral vein thrombosis.** 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 < .001. 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 < .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 identi-
cal in groups I and II at baseline, significantly different at 30
minutes (7.0 ± 1.4 minutes vs 3.6 ± 0.7 minutes, P = .04),
but no longer at 120 minutes (6.1 ± 2.4 minutes vs 3.9 ± 0.8
minutes, P = .4).

**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 thrombo-
lysic 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, notwith-
standing pretreatment with ASA. Indeed, concomitant use
of heparin with rt-PA enhanced venous thrombolysis and
accelerated arterial reperfusion in the dual-thrombosis
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

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

<table>
<thead>
<tr>
<th>Parameter</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>NS</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>&lt;.001</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></td>
</tr>
<tr>
<td>At 150 min</td>
<td>187 ± 2</td>
<td>24 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

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

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**Fig 1. Schematic representation of the femoral arterial perfusion status in two groups of dogs randomized to rt-PA with heparin (group I) or rt-PA without heparin (group II). Black boxes, occluded; gray boxes, patent with blood flow less than 50% of poststenotic flow at baseline; white boxes, patent with blood flow greater than 50% of poststenotic flow at baseline. The numbers represent the sequence number of the experiment.**

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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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