Pharmacokinetics of tissue plasminogen activator in an isolated extracorporeal circuit

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Purpose: The aim of this study was to investigate the pharmacokinetics of tissue plasminogen activator (tPA) under the conditions of an isolated extracorporeal circuit.

Methods: Plasma levels of tPA were measured in the perfusion solution and in central venous blood before, during, and after the perfusion in seven patients undergoing regional hyperthermic fibrinolytic perfusion with tPA in addition to surgical thrombectomy for extended deep venous thrombosis. Results: After 15 minutes of fibrinolytic perfusion, the level of tPA in the perfusion solution was 10,427 ± 4432 ng/mL, and after 30 minutes the maximum level of 19,726 ± 5630 ng/mL was reached. After 60 minutes when the perfusion was discontinued, tPA concentrations dropped to 15,931 ± 4818 ng/mL. In central venous blood, tPA levels increased to a maximum of 230.7 ± 89.6 ng/mL after 60 minutes of perfusion, which represented 1.4% of the concentration measured in the perfusion solution at the same time. With disconnection of the extracorporeal circuit, the tPA levels in central venous blood decreased rapidly and reached a level of 24.1 ± 8.7 ng/mL after 120 minutes.

Conclusion: The use of regional hyperthermic fibrinolytic perfusion in the treatment of extended deep venous thrombosis makes it possible to achieve extremely high concentrations of tPA in the perfusion solution. At the same time, the entry of the fibrinolytic agent into the systemic circulation is minimized. (J Vasc Surg 2001;33:165-9.)

Fibrinolytic therapy is a well-established form of treatment for a variety of diseases, including deep venous thrombosis (DVT). However, because of the possible bleeding complications, fibrinolysis can only be performed in a select group of patients.

With all contraindications taken into account, thrombolytic therapy is feasible in only about 15% of patients with DVT. The restricted use of systemic fibrinolysis has led to the development of alternative techniques; one of them is the regional hyperthermic fibrinolytic perfusion (RHFP) model. Regional perfusion with an extracorporeal circuit was first described by Creech et al in 1958 and has been mainly used in the treatment of advanced melanoma. With isolated fibrinolytic perfusion, residual thrombi after surgical thrombectomy can be addressed intraoperatively with high concentrations of the thrombolytic agent, while at the same time minimizing its systemic levels. Additionally, the temperature in the perfused area can be kept at a higher level; in vitro studies have shown this higher temperature to increase the fibrinolytic activity of tissue plasminogen activator (tPA). The fibrinolytic agent used in the RHFP model, tPA, is removed from the circulation by the liver and has a short half-life of 3 to 5 minutes. Under the conditions of an isolated extracorporeal perfusion with lack of the liver passage, impacts on the pharmacokinetics of tPA had to be expected. We therefore measured the plasma levels of tPA in the perfusion solution and systemic levels before, during, and after the perfusion in seven patients undergoing RHFP.

METHODS

All patients undergoing RHFP were informed explicitly about the procedure and gave written consent. The technique of RHFP has been described previously. After surgical thrombectomy was completed, the common femoral artery and vein of the affected limb were cannulated, and the catheters were connected to a heart-lung machine that contained a bubble oxygenator (Bentley-5; Baxter, Irvine, Calif) (Fig 1). The heart-lung machine was prefilled with 700 mL of gelatine solution (Haemaccel; Behringwerke AG, Marburg/Lahn, Germany) to which 5000 IU of heparin and 20 mval of bicarbonate were added. A rubber tourniquet was wrapped tightly around the groin just above the cannulation site to prevent the entry of the perfusion solution into the systemic circulation through the soft tissue of the leg. A Steinmann pin was introduced into the superior iliac crest and served as an anchor to prevent the tourniquet from sliding distally and obstructing the cannulation catheters. The extremity was wrapped in an aluminum blanket to create heat isolation, and the extracorporeal circuit was initiated. Steady-state hemodynamic conditions within the circuit were established, and the perfusion solution was warmed to 40°C, which took 20 to 30 minutes of perfusion depending on the volume and the initial temperature of the perfused extremity. After that, 0.5 mg/kg of body weight of tPA diluted in 50 mL of 0.9% saline solution was infused through the arterial line of the heart-lung machine over a period of 30 minutes. Simultaneously, 500,000 kallikrein

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inactivation units of aprotinin was infused into the systemic circulation by means of a peripheral venous line. After the infusion of the fibrinolytic agent was completed, the isolated extracorporeal perfusion of the extremity with the tPA-containing solution was kept up for 60 minutes. After completion of the fibrinolytic perfusion, the perfusion solution was directed into a cell-saver, and the leg was perfused with 1000 to 1500 mL of gelatine solution to wash out any remaining intravascular tPA. Afterwards, the leg was filled up with blood that was donated earlier by the patient and the washed cell-saver blood. The venous and arterial catheters as well as the Steinmann pin were removed, and the cannulation sites were closed.

Plasma levels of tPA were measured in the perfusion solution of the heart-lung machine and in central venous blood. The samples from the perfusion solution were obtained from a three-way stopcock placed in the venous line of the heart-lung machine directly before the oxygenator. After the sample was obtained, the line was flushed with 10 mL of sodium chloride, 0.9%. The samples from the systemic circulation were obtained from a central venous line. Five milliliters of blood was drawn before obtaining the sample to avoid dilution effects, and the line was also flushed afterwards with 10 mL of 0.9% saline solution. One sample was taken from the extracorporeal circuit immediately before adding the fibrinolytic agent, and one sample was obtained from the central venous line simultaneously (time 0). Further simultaneous sampling was done 15, 30, 45, and 60 minutes after perfusion start time. Additionally, central venous samples were obtained after disconnection of the extracorporeal circuit and restoration of distal perfusion (time x) as well as after 120 minutes.

The samples were collected in 5-mL EDTA vials (Monovette, Sarstedt, Germany), which were cooled in ice and immediately taken to the laboratory. Ten microliters of PPACK solution (5 mg D-phenylalanyl-2-propyl-L-arginine-methylchloride-ketone diluted in 9.538 mL of 0.1 mmol/L hydrogen chloride solution; pH 4.0) per milliliter
of blood was added. The samples were cooled for 15 additional minutes and then centrifuged. The plasma was pipetted in separate glass tubes and frozen at –20°C. Without an interruption of the cooling process, the samples were taken to a specialized laboratory where tPA concentrations were measured with an immunologic assay (enzyme-linked immunosorbent assay) according to the method described by Seifried et al.14 A two-site microplate enzyme-linked immunosorbent assay with a polyclonal/monoclonal antibody configuration was developed. The plasma samples containing tPA were diluted 1:10 or more in phosphate-buffered saline-bovine serum albumin (PBS-BSA) and incubated (100 µL per well) in the polyclonal antibody-coated microtiter plates for 2 hours at room temperature. After four washes with PBS containing 0.5 g/L Tween 20, 100 µL per well of monoclonal anti-tPA–antibody conjugated to horseradish peroxidase (Boehringer Mannheim, Mannheim, Germany) diluted to 0.006 units peroxidase activity per milliliter in PBS-BSA was incubated for 1 hour. After three further washes with PBS-Tween, solid phase horseradish peroxidase–activity was quantified with incubation for 30 minutes with 100 µL o-phenylene diamine substrate solution followed by 100 µL 2.25 mol/L sulfuric acid and measurement of optical density (A492 nm) with a microplate reader (Dynatech MR 600; Dynatech, Denkendorf, Germany).

The total tPA levels were described as mean value ± SD. The statistical analyses were performed with the Student t test.

RESULTS

Plasma levels of tPA were measured in seven patients. In four (57%) patients, tPA concentrations in the perfusion solution were elevated, ranging between 41.9 and 189.1 ng/mL even before addition of the fibrinolytic agent. The mean tPA level in the perfusion solution before starting fibrinolysis was 68.1 ± 75.7 ng/mL (Table I); the respective level in the systemic circulation was 4.9 ± 1.8 (Table II) and thereby significantly lower (P < .001).

During the 30 minutes when tPA was constantly added to the perfusion solution, a nearly linear increase in the plasma levels of tPA in the perfusion solution was present. After the infusion of tPA was finished, a decrease in tPA plasma levels in the perfusion solution was noted (Table I). The maximum tPA level of 19,726 ± 5630 ng/mL was reached after 30 minutes, and at the end of the perfusion after 60 minutes the mean tPA level in the perfusion solution was 15,931 ± 4818 ng/mL (Table I).

In all seven patients, plasma levels of tPA in the systemic circulation increased during the fibrinolytic perfusion (Table II). The highest plasma level of tPA in the systemic circulation was reached after 60 minutes at the end of the perfusion with 230.7 ± 89.6 ng/mL, showing a continuous increase in systemic tPA levels during the fibrinolytic perfusion. The entry of fibrinolytic agents into the systemic circulation during perfusion is also shown by the relation of systemic tPA levels and tPA levels in the perfusion solution (Fig 2): after 15 minutes of perfusion, the mean systemic tPA level was at 0.7% of the perfusion solution level, after 30 minutes it increased to 0.8%, after 45 minutes it increased to 1.2%, and after 60 minutes at the end of the perfusion it reached 1.4% (Fig 2). At the time of disconnection of the extracorporeal circuit and restoration of perfusion to the limb (time x), the mean tPA level in central venous blood had decreased to 207.7 ± 95.7 ng/mL, and after 120 minutes it had dropped significantly to 24.1 ± 8.7 ng/mL (Table II).

DISCUSSION

Using the RHFP model and infusing 0.5 mg tPA per kilogram of body weight, we could achieve extremely high levels of the thrombolytic agent in the perfusion solution. The concentrations measured in the perfusion circuit were 70 to 100 times higher than the levels of tPA of 200 to 300 ng/mL described in the literature that were achieved with systemic fibrinolysis for DVT.15 With a mean peak level of almost 20,000 ng/mL, the tPA concentrations measured in the RHFP model were also significantly higher than the levels described in systemic fibrinolysis with pulmonary embolism and myocardial infarction.16,17 In the treatment of pulmonary embolism, Tebbe et al16 measured a peak level of 3200 ± 840 ng/mL after bolus injection of 50 mg tPA. That was followed by an infusion of 50 mg tPA over 2 hours, resulting in a mean plasma level of 520 ng/mL. Tanswell et al17 describing fibrinolysis in acute myocardial infarction, applied a bolus of 15 mg tPA followed by an infusion of 50 mg tPA over 30

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Table II. Plasma levels of tPA in the systemic blood before, during, and after the fibrinolytic perfusion

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Patient 1 (ng/mL)</th>
<th>Patient 2 (ng/mL)</th>
<th>Patient 3 (ng/mL)</th>
<th>Patient 4 (ng/mL)</th>
<th>Patient 5 (ng/mL)</th>
<th>Patient 6 (ng/mL)</th>
<th>Patient 7 (ng/mL)</th>
<th>Total (ng/mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0‡</td>
<td>5.4</td>
<td>4.9</td>
<td>2.5</td>
<td>8.1</td>
<td>3.3</td>
<td>5.5</td>
<td>4.8</td>
<td>4.9 ± 1.8</td>
</tr>
<tr>
<td>15</td>
<td>7.8</td>
<td>205.6</td>
<td>107.5</td>
<td>86.6</td>
<td>7.5</td>
<td>50.3</td>
<td>34.9</td>
<td>71.5 ± 70.0</td>
</tr>
<tr>
<td>30</td>
<td>30.9</td>
<td>292.2</td>
<td>85.8</td>
<td>302.1</td>
<td>115.7</td>
<td>193.4</td>
<td>117.3</td>
<td>162.6 ± 104.0</td>
</tr>
<tr>
<td>45</td>
<td>119.9</td>
<td>308.3</td>
<td>219</td>
<td>181.1</td>
<td>103.8</td>
<td>341.5</td>
<td>254.1</td>
<td>218.2 ± 90.1</td>
</tr>
<tr>
<td>60</td>
<td>151.4</td>
<td>395.2</td>
<td>193.1</td>
<td>296.5</td>
<td>185.9</td>
<td>147.9</td>
<td>245.9</td>
<td>230.7 ± 89.6</td>
</tr>
<tr>
<td>x‡</td>
<td>274.5</td>
<td>216.4</td>
<td>339.9</td>
<td>199.8</td>
<td>87.9</td>
<td>80.1</td>
<td>255.5</td>
<td>207.7 ± 95.7</td>
</tr>
<tr>
<td>120</td>
<td>333</td>
<td>26.4</td>
<td>24.5</td>
<td>9</td>
<td>32.4</td>
<td>16.2</td>
<td>27</td>
<td>24.1 ± 8.7</td>
</tr>
</tbody>
</table>

*Total tPA levels were calculated as the mean value ± SD.
†Time 0 is the point immediately before administration of tPA to the perfusion solution.
‡Time x is the time at which the perfusion catheters were removed and blood flow to the limb was restored.
They measured a peak level of 3200 ± 840 ng/mL. In a study with healthy volunteers, Seifried et al\textsuperscript{14} applied systemically 0.5 mg tPA per kilogram of body weight, the same dose we used in the RHFP model, and achieved peak plasma levels of about 2000 ng/mL.

In recent years, local, catheter-directed thrombolysis has replaced the systemic therapy in many instances. With the catheter-directed technique, the thrombolytic agent is administered directly into the thrombus, thereby decreasing the total dose required for therapy and the systemic drug concentration.\textsuperscript{18} Although there are numerous studies in which the clinical results of catheter-directed thrombolysis are addressed,\textsuperscript{18} there are few data regarding the pharmacokinetics and the local and systemic levels of tPA reached with this technique. Rauber et al\textsuperscript{19} described systemic tPA levels of 60.3 ± 4.3 ng/mL after catheter-directed application of 20 mg of tPA in patients with peripheral arterial occlusive disease. Higher dosages of up to 100 mg of tPA have been used in local thrombolysis,\textsuperscript{20} with no information being provided about what tPA levels were achieved. To our knowledge, there is no study in which the pharmacokinetics and systemic and local levels of tPA in systemic versus catheter-directed thrombolysis are compared.

The peak plasma levels of about 20,000 ng/mL, which were reached with the RHFP model, are, to our knowledge, the highest levels achieved in the clinical application of tPA. The high plasma levels are a result of the high initial dose of tPA and of the altered pharmacokinetics in the isolated circuit. Because the distribution volume for tPA is smaller and the elimination of tPA in the liver is avoided in isolated perfusion, the tPA levels had still remained at 80% of the peak level at the end of the perfusion. This is in contrast to systemic fibrinolysis, where tPA has only a short half-life and is eliminated from the circulation rapidly.\textsuperscript{14}

Even though the use of an isolated, extracorporeal circuit diminishes the entry of tPA into the systemic circulation, it cannot avoid it totally. During the perfusion, the tPA levels in the systemic circulation increased constantly and reached 230 ng/mL after 60 minutes (Table II). After 15 minutes of perfusion, the systemic tPA concentration was at 0.7% of the concentration measured in the perfusion solution; after 60 minutes it had approached 1.4% (Fig 2). At the time of disconnection of the extracorporeal circuit the systemic levels had decreased to 207.7 ng/mL, and after 120 minutes the concentration had dropped significantly to 24.1 ng/mL (Table II). Accordingly, systemic levels of tPA of 200 to 230 ng/mL were maintained for about 30 to 45 minutes. Although these levels are lower than the concentrations used in systemic thrombolysis, they are 40 to 50 times higher than the baseline tPA levels measured in the patients (Table II). Because a strong dose-effect relationship has been demonstrated for tPA,\textsuperscript{21} this could potentially lead to systemic bleeding complications despite the use of the isolated extracorporeal circuit. On the other hand, it has been shown that the application of lower concentrations of tPA over a relatively short period

![Fig 2. Relation between levels of tPA in central venous blood and in perfusion solution. Relative concentration of tPA in systemic blood was calculated as tPA levels in central venous blood divided by respective tPA levels in perfusion solution times 100. It increased during perfusion and after 60 minutes reached 1.4% of concentration in perfusion solution. tPA, Tissue plasminogen activator.](image-url)
of time is probably of little clinical significance, especially if high concentrations of plasmin inhibitors are present.\textsuperscript{22,23} The antiplasmin pool of the patients’ blood can be artificially elevated with the infusion of aprotinin, which inhibits plasmin and thereby effectively counters the effects of tPA.\textsuperscript{24-26} Clinically, aprotinin has been shown to reduce blood loss in patients undergoing cardiopulmonary bypass grafting.\textsuperscript{27} The combination of relatively low systemic tPA levels (200-230 ng/mL) with a short application time of 30 to 45 minutes in addition to artificially elevated antiplasmin levels in the patients’ blood should decrease the risk of unwanted systemic effects of tPA.

In conclusion, extremely high concentrations of tPA can be achieved in the perfusion solution with the use of RHFP; at the same time the systemic levels of the thrombolytic agent can be minimized. The clinical results of this method must be evaluated before any conclusions can be drawn regarding its possible role as a supplement to surgical thrombectomy in the treatment of extended DVT.

REFERENCES