Aprotinin: An Antidote for Recombinant Tissue-Type Plasminogen Activator (rt-PA) Active In Vivo

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The goal of the study was to assess if aprotinin, a protease inhibitor, could be used to antagonize in vivo the effects of recombinant tissue-type plasminogen activator (rt-PA). The time course of the lysis of a radioactive jugular vein thrombus was monitored continuously with an external gamma counter in anesthetized rabbits. Recombinant rt-PA (0.25 mg) was given intravenously as a bolus injection (10% of the dose), followed by a 4 h infusion (90% of the dose). Rabbits received aprotinin 20 min after the start of the infusion as an intravenous bolus injection at a dose of 60,000 IU/kg (n = 4) or 20,000 IU/kg (n = 4).

Recombinant tissue-type plasminogen activator (rt-PA) is a potent thrombolytic drug that is effective in patients with myocardial infarction (1-4). Its use has been associated with major bleeding complication rates of 8% (3), 15% (6) and 17% (2). Bleeding complications such as cerebral hemorrhage can be life-threatening and with the increasing use of rt-PA, their absolute number will increase.

Until recently, no antidote for rt-PA-induced bleeding had been described. It was always thought that in case of hemorrhagic complications, stopping rt-PA administration was sufficient because the half-life of rt-PA is short (7). However, it has been shown that in vivo the rate of thrombolysis is not parallel to the plasma concentration of rt-PA. Thrombolysis can continue without any measurable rt-PA concentration in plasma (8). This is one reason why the use of a recombinant plasminogen activator inhibitor to antagonize the effects of rt-PA was recently proposed (9). This specific inhibitor of rt-PA reduced the rt-PA-induced increase in bleeding time in rabbits. To our knowledge, the clinical use of this inhibitor has not been described.

In contrast, aprotinin is a protease inhibitor that has been used in patients with acute pancreatitis, abnormal blood loss and shock (10). In vitro, aprotinin blocks the thrombolytic effect of plasmin and, in this way, the thrombolytic effects of plasminogen activators (11). However, to our knowledge the antagonizing effects of aprotinin on rt-PA-induced thrombolysis have not been tested in vivo.

We recently modified a previously described model (8) to continuously monitor the lysis of a jugular vein thrombus in anesthetized rabbits (8). In the present study, we assessed the effects of aprotinin during administration of rt-PA in this model. Moreover, because the bleeding time is a relatively good predictor of hemorrhagic events secondary to administration of rt-PA (12), we evaluated the effects of rt-PA on the prolongation of bleeding time.

Methods

Thrombolysis experiments. Burgundy rabbits weighing 2.5 to 3 kg were anesthetized by intravenous injection of pentobarbital (35 mg/kg body weight) (Vetalarol, Veterinaria AG). Additional pentobarbital was given when needed to maintain anesthesia. The rabbit was tracheotomized and its lungs were ventilated by a mixture of 50% room air and 50% oxygen. A catheter was introduced into a femoral vein for influsing drugs and another catheter was implanted into the femoral artery for blood sampling.

An artificial thrombus was produced in the jugular vein as described by Colten et al. (12). Briefly, the external jugular
Liquemin, F. Hoffmann-La Roche) was given intravenously through the facial vein catheter. The clot was aged for 30 min before the introduction of fresh rabbit blood and 10 IU of thrombin (F. Hoffmann-La Roche) and injected into the jugular vein segment through a polyethylene catheter. Indium-125-labeled human fibrinogen (Amersham) containing approximately 800,000 counts/min was mixed with the blood of all rabbits by suction through the facial vein catheter. i.v. = intravenously.

Thrombolysis was evaluated by continuously monitoring the decrease in thrombus radioactivity as a measure of the decrease in thrombus size. For this purpose, a gamma detector (model 8 SHAIM/2, Harsaw) connected to a multichannel analyzer (model ND 62, Nuclear Data) was positioned 2 cm over the jugular vein and a few minutes after introduction of the radioactive clot into the isolated jugular vein segment. Radioactivity was continuously recorded with a counting time of 45 s (Fig. 1). The decrease in radioactivity was displayed on the multichannel analyzer and recorded on a floppy disk. Stability of the clot was assessed by measuring the radioactivity for 15 min after release of the clamps. At the end of the experiments, residual thrombus was removed and the background radioactivity of the remaining preparation was measured with the detector in the same position as during the thrombolysis experiment.

Protocol. Four groups of rabbits were studied. One group of four rabbits was given only the rt-PA vehicle and was used as the control group. The three other groups received rt-PA (0.25 mg/kg) given as a bolus injection (10% of the total dose) followed by a continuous infusion (90% of the total dose) over 4 h. Among these three groups, one received only rt-PA (n = 6) and the two others received aprotinin (20,000 IU/kg, n = 4 or 60,000 IU/kg, n = 4) given as a bolus injection 20 min after the start of rt-PA administration. With this design, it was possible to determine whether the thrombus could be lysed during the first 20 min of the study. To assess the effects of aprotinin, we compared the rate of lysis in the different groups 2, 60 and 180 min after administration of aprotinin.

To estimate the rate of lysis, curve fitting was done for each rabbit separately. The counts were adjusted for the initial and the background radioactivity. Only data measured after the time of aprotinin administration (20 min) were used. For rabbits that did not receive aprotinin, a nonlinear regression model (PROC NLIN, SAS Institute) with an exponential decrease was used:

\[
T(t) = A e^{-\gamma t} + L,
\]

where \(T\) is the thrombus size in percent, \(A\) is the lysable fraction, \(L\) is the theoretically unlysable fraction, \(t\) is time and \(\gamma\) is the lysis rate. For rabbits that received aprotinin, a cubic polynomial (PROC REG, SAS Institute) was fitted to the data.

At 2, 60 and 180 min after administration of aprotinin, the lysis rate relative to the remaining thrombus at that time was calculated from the estimated regression variables. The effect of the different dosing regimens on the relative lysis rate was compared using a weighted one-way analysis of variance (PROC GLM, SAS Institute) with the factor dose group. Weights were the inverses of the variances of the relative lysis rate in each group.

Bleeding experiments. Two groups of rabbits were anesthetized and ventilated as in the thrombolysis experiments. Template bleeding time was measured by making a 1 mm incision in the left ear of the rabbit with a lancet (Autodisc lancet, Boehringer). The ear of the rabbit was maintained on a surface of water kept at 37°C and the end of bleeding was determined by looking at the blood dropping in water.

Bleeding time was determined at baseline, 5 and 10 min after administration of heparin (100 IU/kg) and 5 min after injection of rt-PA (0.5 mg/kg followed by 0.5 mg/kg per h).
Table 1. Effects of Aprotinin on Thrombolysis Induced by rt-PA

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>No.</th>
<th>Thrombolysis Rate (% of remaining thrombus/min)</th>
<th>2 Min</th>
<th>60 Min</th>
<th>180 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>rt-PA</td>
<td>6</td>
<td>0.012 ± 0.006</td>
<td>0.007 ± 0.007</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>rt-PA + aprotinin</td>
<td>4</td>
<td>0.076 ± 0.016</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>(30,000 IU/kg)</td>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>rt-PA + aprotinin</td>
<td>4</td>
<td>0.033 ± 0.061</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>(60,000 IU/kg)</td>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
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</table>

*p < 0.05 versus control group; †p < 0.01 versus control group; ‡p < 0.001 versus control group; †p < 0.01 versus recombinant tissue-type plasminogen activator (rt-PA) group; ‡p < 0.001 versus rt-PA group. Thrombolysis rate was determined after aprotinin injection.

Results

Thrombolysis experiments. In the control group that did not receive rt-PA, the rate of thrombolysis was very slow (Fig. 1). In contrast, administration of rt-PA induced rapid thrombolysis with an exponential shape. The bolus injection of aprotinin dramatically slowed thrombolysis in a dose-dependent fashion. The rate of lysis in the four experimental groups is given in Table 1. At 2 min after administration of aprotinin, the rate of thrombolysis was equal to the rate of thrombolysis observed in the control group that did not receive rt-PA. At 60 min after injection of aprotinin, the rate of thrombolysis was still decreased with both doses. However, at 180 min after administration of aprotinin, the rate of lysis was decreased only in the high dose group (60,000 IU/kg of aprotinin).

Bleeding time experiments. Bleeding time increased approximately 100% after administration of heparin and by >100% with rt-PA (Fig. 2). Aprotinin completely prevented this increase in bleeding time.

Discussion

Effects of aprotinin on thrombolysis induced by rt-PA. These results show that in vivo aprotinin can block the effects of rt-PA. Moreover, using an external gamma counter, we were able to show that the effect of aprotinin has a very short onset and is long-lasting.

Aprotinin is a protease inhibitor that inhibits plasmin. Its clinical indications were recently reviewed (10). Aprotinin has been used in patients with acute pancreatitis, blood loss and shock (10). In vitro, it was shown (11) that aprotinin could block the effects of rt-PA. Our results confirm that aprotinin can also be used in vivo.

The onset of action of aprotinin is very short. This was an interesting point to check because an antidote for rt-PA-induced bleeding could be useful only if it has a very short onset of action; rt-PA has a short half-life (approximately 3 min) in rabbits (7). Thus, cessation of the infusion of rt-PA leads to a rapid decline in its plasma concentration (7). However, we recently showed (8) that the time course of the thrombolysis is not parallel to the time course of the plasma concentration of rt-PA. Cessation of the rt-PA infusion leads to a nearly complete arrest of thrombolysis in approximately 1 h (8). Thus, the advantage of aprotinin is its immediate antagonism of rt-PA.

Another interesting point is that the effect of aprotinin was rather long-lasting, allowing its administration as a bolus dose. However, our results show that the duration of action of aprotinin is clearly dose dependent. A dose of 60,000 IU/kg is needed to obtain a 3 h duration of action.

Effects of aprotinin on the rt-PA-induced increase in bleeding time. Bleeding time was recently shown (13) to be correlated with bleeding complications during treatment of acute myocardial infarction. Thus, it was important to show that aprotinin not only could stop the thrombolytic effect of rt-PA, but also prevent the increase of bleeding time due to rt-PA. Recently, recombinant plasminogen activator inhibitor 1 was shown (9) to reverse the bleeding tendency.
associated with the combined administration of rt-PA and aspirin in rabbits. In that study, tranexamic acid was significantly less potent than the rt-PA inhibitor in reversing the bleeding tendency, but only one dose was used.

Clinical implications. In the present study, aprotinin could completely prevent the increase in bleeding time due to rt-PA; therefore, no improvement in efficacy could be expected by using the plasminogen activator inhibitor. However, the main advantage of using this inhibitor is that it is believed to be specific. Aprotinin is a nonspecific series protease inhibitor; it inhibits the effects of rt-PA by inhibiting plasmin. Its main advantage is that in contrast to the plasminogen activator inhibitor, it has been used in patients (10). Moreover, the drawbacks due to its nonspecific effects are not clear. Thus, further studies should compare the effects of aprotinin and rt-PA inhibitor in preclinical experiments to determine which is the most suitable compound for clinical testing.

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References