Thrombolytic Treatment of Clot Embolism in Rat
Comparison of Intra-arterial and Intravenous Application of Recombinant Tissue Plasminogen Activator

Frank Niessen, MD; Thomas Hilger, PhD; Mathias Hoehn, PhD; Konstantin-A. Hossmann, MD, PhD

Background and Purpose—We sought to test the hypothesis that intra-arterial recombinant tissue plasminogen activator (rtPA) treatment of thromboembolic stroke is more efficient than intravenous application.

Methods—Rats were embolized by intracarotid injection of autologous fibrin-rich blood clots. One hour later rtPA (10 mg/kg) was infused either intravenously (n=8) or intra-arterially (n=8). Control rats (n=8) received intra-arterial infusion of saline. Treatment was monitored by MR perfusion-weighted imaging and apparent diffusion coefficient (ADC) imaging, and outcome was evaluated by comparing incidence of hemorrhages and lesion volumes of ATP and pH.

Results—Clot embolism led to a decline of perfusion-weighted imaging signal intensity in the middle cerebral artery territory to <40% of control. Both intra-arterial and intravenous treatment significantly improved blood flow in cerebral cortex but not in caudate putamen. In untreated animals, ATP and pH lesion volumes were 510.3±94.5 and 438.6±39.2 mm³ at 7 hours after clot embolism, respectively. Both intravenous and intra-arterial rtPA treatment produced hemorrhagic complications but reduced ATP lesion size to 296.2±136.1 and 370.3±103.7 mm³ and reduced pH lesion size to 263.3±114.6 and 303.3±103.0 mm³, respectively (P<0.05 for untreated versus treated rats; no difference between intravenous and intra-arterial treatment). ADC imaging revealed that lesion reduction was due to inhibition of infarct growth but not to reversal of primary injury.

Conclusions—This study documents reduction of injury by rtPA treatment but does not reveal a difference between intra-arterial and intravenous application. Our data do not support an advantage of intra-arterial thrombolysis. (Stroke. 2002;33:2999-3005.)

Key Words: embolism ■ magnetic resonance imaging ■ thrombolytic therapy ■ rats

The positive outcome of the National Institute of Neurological Disorders and Stroke (NINDS) rt-PA Stroke Study Group on the intravenous treatment of acute ischemic stroke with tissue plasminogen activator (tPA) has led to the increasing use of this drug under general clinical conditions. Although later controlled trials with less stringent inclusion criteria were not able to replicate the statistical difference between treated and untreated patients, post hoc analysis of patient subgroups that matched the original NINDS protocol confirmed the improved chances of treated stroke patients for favorable outcome. Similar observations were made in several open trials that were performed in accordance with the NINDS treatment protocol. Thrombolysis is therefore widely accepted as a stroke treatment with proven therapeutic benefit.

Despite these encouraging observations, thrombolytic treatment calls for further improvement. The basic requirement to initiate therapy within <3 hours after stroke onset and the difficult diagnostic procedures necessary to exclude patients with increased risk of bleeding limit the number of eligible patients to <15%, even in centers with highly developed logistics for rapid initiation of stroke therapy. Because treatment of these patients increases favorable outcome by only 13%, the number of stroke victims who profit from this therapy narrows to <2%. Obviously, a positive outcome of thrombolysis depends critically on the speed and efficacy of postthrombotic recanalization. It has been suggested that recanalization can be accelerated by intra-arterial recombinant tPA (rtPA) application, but experimental studies clearly demonstrate that even with the use of this route, thrombolytic reperfusion is a slowly progressing process. This raises the question of whether the faster rise of the drug concentration at the clot site achieved by intra-arterial compared with intravenous application is of any pathophysiological relevance for flow restoration. On the other hand, intra-arterial application carries a higher risk of procedural complications, and the increased blood-to-brain transfer of tPA may induce neurotoxicity. We therefore investigated whether intra-arterial infusion actually improves outcome compared with intravenous application.
Materials and Methods

Animal Model
All experiments were performed in accordance with the National Institutes of Health Animal Protection Guidelines and were approved by the local government authorities.

Male Wistar rats weighing 300 to 400 g were anesthetized with 2% isoflurane in a 2:1 mixture of N2O/O2. Rectal temperature was kept constant at 37°C with the use of a feedback-controlled heating blanket. The right femoral artery and vein were catheterized for blood pressure measurement, blood sampling, and injection of drugs. Animals were tracheotomized, immobilized with pancuronium bromide (0.3 mg/kg per hour), and mechanically ventilated. Isoflurane concentration was then reduced to 1.5%. Blood gases were measured every hour and kept within physiological range. The right pterygopalatine and occipital arteries were ligated. A PE50 catheter was inserted into the right external carotid artery (ECA) and continuously rinsed with normal saline at a rate of 1 mL/h to prevent catheter obstruction. The head of the animal was fixed in a stereotaxic head holder and positioned in the center of a 4.7-T horizontal magnet for MRI.

Twelve autologous fibrin-rich blood clots were prepared as described before 12 and drawn into 1-m-long PE50 tubing that was connected to the ECA catheter. The number of clots corresponds to the previously determined amount of clot material required to produce consistent vascular occlusion in untreated and successful recanalization in rtPA-treated rats.12

Experimental embolic stroke was induced inside the magnet by injecting the 12 clots over 30 seconds into the carotid circulation. While the injection was performed, the right common carotid artery was temporarily occluded with a remotely controlled occlusion device.9 One hour after clot embolism, animals were randomly divided into 3 groups, receiving either no treatment or intra-arterial or intravenous rtPA infusions. Intravenous treatment (n=8) was performed by dissolving rtPA (10 mg/kg) in 2 mL distilled water and applying 10% of the solution as a bolus and the rest as a 1-hour infusion into the femoral vein. Intra-arterial treatment (n=8) was done in the same way by infusing the drug through the ECA catheter into the ipsilateral carotid artery. Untreated animals (n=8) were infused with 2 mL saline into the ECA catheter. In a manner similar to that used in the treated animals, 10% of the fluid was given as a bolus and the rest as constant infusion over 1 hour.

Magnetic Resonance Imaging
Nuclear MR measurements were performed at 200 MHz with the use of a Bruker BioSpec system (Bruker Medical) with a 4.7-T horizontal magnet of 30-cm bore. The system was equipped with actively shielded gradient coils (maximum gradient strength 100 mT/m; gradient rise time <250 μs). Diffusion-weighted MRI was performed with a multislice Stejskal-Tanner-type spin-echo sequence.13 The sequence parameters were as follows: echo time=35.2 ms, repetition time=3235 ms, matrix=128×128. Six coronal slices with a thickness of 1.21 mm and an interslice gap of 0.54 mm were recorded with a field of view of 4×4 cm.2. For the quantitative determination of the apparent diffusion coefficient (ADC), diffusion-weighted MRIs with different gradient strengths (b values: 30, 100, 400, 1000, 2000 s/mm²) were recorded.

Single-slice perfusion-weighted images through the center of the middle cerebral artery (MCA) territory (ie, at the level of the caudate putamen) were obtained with the use of a modification14 of the arterial spin-tagging technique.15 During the first acquisition, arterial spins flowing through the neck were inverted adiabatically (tagging), and the inflow of labeled spins was detected with a snapshot fast low-angle shot imaging (FLASH) sequence (echo time=3.9 ms, repetition time=7.4 ms, matrix=128×64, slice thickness=2 mm, field of view=4×4 cm²). The second acquisition left the inflowing spins undisturbed by changing the sign of the frequency offset (untagged image). Eight tagged and untagged images each were averaged to improve the signal-to-noise ratio. Perfusion-weighted images were calculated by subtraction of the tagged from the untagged images and normalization to the untagged images to compensate for signal loss in regions more distal to the receiver surface coil.

An ADC multislice set and a perfusion-weighted image at the level of the caudate putamen were obtained before embolism and at 1, 2, and 6 hours after embolism.

Image Analysis
ADC maps and normalized perfusion-weighted images were transferred to a Macintosh Power PC 7200/66 (Apple) and submitted to image analysis with the use of the image processing software IMAGE (National Institutes of Health).

Data analysis was performed in individual voxels. Relative ADC and relative perfusion signal intensities were calculated pixelwise by dividing ADC and perfusion values by the corresponding preembolic data. The ischemic lesion was defined as the decline of ADC to <80% of control because this threshold correlates with the loss of ATP both during ischemia16 and during the initial 10 hours of posts ischemic reperfusion.17

The hemispheric lesion volume (HLV) was calculated by multiplying the sum of the lesion areas for all slices with the interslice distance and was corrected for edema by subtracting the nonischemic part of the lesioned hemisphere from the opposite hemisphere.18 Signal intensities of perfusion-weighted images were measured in regions of interest placed in the lateral caudate putamen and the parietal cortex. Signal intensities were expressed as percentage of preischemic control.

ATP and pH Imaging
Brains were frozen in situ at 7 hours after embolism, removed from the skull in a cold box at −20°C, and sliced into 20-μm sections with a cryostat microtome. Coronal sections corresponding to the 6 levels of ADC slices were processed for the regional distribution of ATP by evoking substrate-specific bioluminescence.19 Regional tissue pH was measured in coronal sections adjacent to the ATP images with the umbelliferone fluorescence technique.20 ATP lesion volume was defined as the region with a decline of ATP-induced bioluminescence by >2 SD below the mean contralateral value, and pH lesion volume was defined as a decline to <6.3.

Histology
Cryostat sections were stained with hematoxylin-eosin and inspected for intracerebral hemorrhages. The severity of hemorrhages was scaled as described in Table 2.

Statistical Analysis
The number of animals per group was set at n=8 on the basis of the following assumptions. The expected scatter of mean values in this experiment is approximately 25%, and the expected relative difference in lesion size between intra-arterial and intravenous treatment is approximately 35%.8 At a statistical power of 0.7, the minimum difference in lesion size between intra-arterial and intravenous treatment is approximately 35%.8 At a statistical power of 0.7, the minimum sample size for a CI of 95% is n=8. Measurements were performed by a person blinded to the experimental protocol and are presented as mean±SD. Physiological variables, MR data, and ATPPH lesion volumes were compared by 1-way ANOVA, with the Scheffe test for multiple comparisons between groups. The incidence of cerebral hemorrhages was assessed by the Kruskal-Wallis test followed by the Mann-Whitney U test. Differences were considered to be significant at P<0.05. Statistical analysis was performed with the software package StatView for Windows (release 5.0.1, SAS Institute, Inc).

Results

General Physiological Observations
Twenty-four of 31 animals submitted to intracarotid clot embolism were exposed to the full experimental protocol. The other experiments were terminated earlier: 2 experiments because of failure in temperature or respiration control and 5 experiments because of uncontrollable bleedings from the
Untreated embolism (n = 8)

The CAudate putamen was 33.5% of the perfusion-weighted imaging signal intensity in the ipsilateral hemisphere (Figure 1). Clot embolism through the right carotid artery led to a marked decline of perfusion-weighted imaging signal in the left MCA territory. Infusion of clots into the right carotid artery led to a marked decline of perfusion-weighted imaging signal intensity in the ipsilateral hemisphere (Figure 1).

Perfusion-Weighted Imaging

Before clot embolism, perfusion-weighted imaging exhibited symmetrical distribution of signal intensity in both hemispheres. In untreated animals, physiological variables were within normal range throughout the observation period of 7 hours, and there was no difference between the 2 treatment groups (Table 1).

ADC Imaging

Preembolism ADC maps did not exhibit asymmetries between the 2 hemispheres. Embolism led to a pronounced reduction of ADC in the center of the ipsilateral MCA territory and, to a lesser degree, in the more peripheral zones as well. One hour after embolism, the HLVs in which ADC declined to <80% of control did not differ between groups (495.1 ± 119.7, 457.4 ± 130.2, and 566.8 ± 55.3 mm³ in the untreated, intravenous, and intra-arterial treatment groups, respectively; P = NS). At the end of the observation time, 6 hours after embolism, the lesion volumes were 580.3 ± 136.8, 471.3 ± 130.6, and 594.4 ± 77.0 mm³, respectively. These values correspond to a significant lesion growth during this time by 18.2 ± 16.3% in the untreated animals (P < 0.05) but an insignificant change by 4.4 ± 18.0% after intravenous treatment and by 5.6 ± 16.4% after intra-arterial treatment (Figure 3). rtPA treatment thus stabilized the lesion size at the pretreatment level, irrespective of the route of application.

Biochemical Imaging

Biochemical lesion volumes were measured at the end of the experiments, at 7 hours after embolism, which was 6 hours after onset of treatment. In the untreated group, the HLV of ATP depletion was 510.3 ± 94.5 mm³ (Figure 3). Intra-arterial rtPA treatment resulted in a significant reduction of the HLV.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MABP, mm Hg</th>
<th>Heart Rate, bpm</th>
<th>Temperature, °C</th>
<th>pH</th>
<th>PCO₂, mm Hg</th>
<th>PO₂, mm Hg</th>
<th>Serum K⁺, mmol/L</th>
<th>Hematocrit, Fraction</th>
<th>Glucose, mmol/L</th>
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<tr>
<td>Untreated embolism (n = 8)</td>
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<tr>
<td>Control</td>
<td>121 ± 8</td>
<td>399 ± 15</td>
<td>36.6 ± 0.4</td>
<td>7.38 ± 0.04</td>
<td>44 ± 5</td>
<td>136 ± 4</td>
<td>4.7 ± 0.6</td>
<td>0.42 ± 0.02</td>
<td>6.8 ± 1.0</td>
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<tr>
<td>1-h embolism</td>
<td>119 ± 8</td>
<td>392 ± 13</td>
<td>36.7 ± 0.4</td>
<td>7.39 ± 0.05</td>
<td>42 ± 3</td>
<td>135 ± 6</td>
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<td>0.41 ± 0.02</td>
<td>6.3 ± 1.3</td>
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<tr>
<td>7-h embolism</td>
<td>101 ± 5</td>
<td>395 ± 11</td>
<td>37.0 ± 0.2</td>
<td>7.38 ± 0.03</td>
<td>42 ± 6</td>
<td>138 ± 7</td>
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<tr>
<td>Control</td>
<td>119 ± 11</td>
<td>406 ± 19</td>
<td>37.2 ± 0.3</td>
<td>7.40 ± 0.06</td>
<td>40 ± 3</td>
<td>131 ± 7</td>
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<td>6.1 ± 1.4</td>
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<td>0.38 ± 0.02</td>
<td>6.1 ± 1.7</td>
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MABP indicates mean arterial blood pressure. Values are mean ± SD.

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<tr>
<td>Untreated embolism (n = 8)</td>
<td>7</td>
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<tr>
<td>Intravenous rtPA (n = 8)</td>
<td>0</td>
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<tr>
<td>Intra-arteral rtPA (n = 8)</td>
<td>0</td>
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Brain sections were graded for hemorrhage as follows: 0, no hemorrhage; 1, single microscopically visible hemorrhage; 2, multiple microscopically visible hemorrhages; 3, macroscopically visible non–space-occupying hemorrhage; 4, macroscopically visible space-occupying hemorrhage. The difference between untreated and treated groups was statistically significant (1-sided Mann-Whitney U test, P < 0.0001) but not that between intravenous and intra-arterial rtPA treatment.
of ATP depletion to 296.2±136.1 mm³ (P<0.05), and intra-arterial rtPA treatment resulted in a significant reduction to 370.3±103.7 mm³ (P<0.05). The difference between the 2 treatment groups was not significant.

Measurements of the HLV representing tissue acidosis led to similar results. In the untreated animals acidosis was present in 438.6±39.2 mm³. pH lesion volume declined to 263.3±114.6 mm³ in the intravenous rtPA treatment group and to 303.3±103.0 mm³ in the intra-arterial rtPA group. The difference between the treated and untreated groups was significant (P<0.05), but that between the 2 treatment paradigms was not significant.

Figure 1. MRI of the brain before and after MCA clot embolism, showing comparison of untreated animal with animals treated at 1 hour after embolism by intravenous or intra-arterial infusion of 10 mg/kg rtPA. PWI indicates perfusion-weighted imaging; ADC, mapping of the apparent diffusion coefficient of water; and pH and ATP, biochemical images of cryostat sections prepared after the last acquisition of MR data.

Figure 2. Changes in signal intensity (SI) of perfusion-weighted MR images in caudate putamen (left) and parietal cortex (right), showing comparison of untreated animals with animals treated at 1 hour after embolism by intravenous or intra-arterial infusion of 10 mg/kg rtPA. Both treatments significantly improved blood flow in cerebral cortex but not in caudate putamen. Differences between the 2 treatment groups were insignificant (*P<0.05, **P<0.01).
Brain Hemorrhage
Histological examination of the brains revealed a marked difference between treated and untreated animals. In the untreated group, 7 of 8 animals did not exhibit any bleeding, and only 1 animal showed a single microscopically detectable hemorrhage (Table 2). In contrast, all of the treated animals suffered hemorrhages, and 1 in each treatment group had space-occupying bleeding. This difference was statistically highly significant (P<0.001). Between the 2 treatment groups, no difference could be detected.

Discussion
Our investigation on the effect of intra-arterial and intravenous rtPA treatment of MCA clot embolism in rats led to 3 main conclusions: (1) a high dose of tPA applied at 1 hour after embolism improves brain perfusion only in the periphery but not in the core of the MCA territory; (2) despite its limited hemodynamic effects, rtPA reduces ischemic damage by preventing injury expansion; and (3) intra-arterial or intravenous application of rtPA has the same therapeutic effect. These observations are discussed separately.

Hemodynamic Effects of rtPA
The incomplete reversal of tissue perfusion by rtPA was unexpected because previous studies repeatedly reported successful recanalization under both experimental and clinical conditions. However, most of these reports documented recanalization at later time points than studied in the present investigation. There may also be a dissociation between recanalization and restoration of tissue perfusion. In fact, prolonged vascular occlusion may provoke a no-reflow phenomenon, which has been attributed to microvascular compression by swollen endothelial and perivascular glial cells and to the increase in blood viscosity. rtPA improves blood flow by reducing blood viscosity, but at low flow values this effect is less pronounced because it cannot prevent cellular swelling and hence microvascular compression. This is presumably the reason that rtPA improves blood flow in the penumbra but does not restore circulation in the core of infarction.

rtPA-Induced Reduction of Tissue Injury
Despite its limited hemodynamic effect and the increased incidence of hemorrhagic transformations, rtPA delivery reduced the severity of the ischemic lesion, as reflected by the smaller volumes of ATP-depleted and severely acidic brain tissue. Interestingly, the mean ATP lesion volume measured at the end of the experiments was larger than the pH lesion volume. This is different from the early stages of permanent MCA occlusion in which the pH lesion is consistently larger because of the evolution of penumbral acidosis. This difference results from secondary pH changes in peri-infarct surroundings, induced either by improvement of blood flow or by rebound alkalization after recovery from peri-infarct spreading depression.

The evaluation of the dynamics of the ADC changes confirmed our earlier observation that the reduction of the biochemical lesion volume is not due to the reversal of the initial ischemic injury but to the prevention of infarct growth. This explains why the therapeutic efficacy declines with increasing treatment delay. In fact, comparison of ADC recordings after different treatment delays revealed that rtPA freezes the lesion volume at the level it has reached when treatment begins. Because up to this time ADC volume steadily expands, the salvageable tissue mass declines accordingly.

The most likely mechanism for infarct expansion in untreated animals is the penumbral mismatch between reduced blood flow and intermittently occurring metabolic challenges, notably peri-infarct spreading depressions (for review, see Hossmann). rtPA-induced improvement of blood flow in the peri-infarct penumbra reduces this mismatch and therefore prevents secondary infarction.

Compared with the beneficial effects of flow improvement, rtPA-induced neurotoxicity seems to be of lesser importance. In fact, previous studies in tPA-deficient animals suggest that tPA neurotoxicity is of pathophysiological relevance only under conditions in which tPA does not improve blood flow, eg, in the absence of endothelial fibrin deposits or cerebrovascular thrombosis. In the present situation of thromboembolic stroke, the beneficial hemodynamic effect of rtPA in the peri-infarct penumbra was therefore the dominant drug action.

Intra-arterial Versus Intravenous Application
Similar to findings of an earlier study of prourokinase, we found that intra-arterial rtPA application was not superior to the intravenous route. Our results are also in accordance with
a study in rabbits that failed to demonstrate a difference in 2,3,5-triphenyltetrazolium chloride staining, but they do not support clinical observations that suggest superiority of intra-arterial rtPA application. Interestingly, there was also no difference in bleeding complications.

It is unlikely that the absence of a difference between intra-arterial and intravenous application is a falsely negative result. The sample sizes of the treatment groups were chosen for a statistical power of 0.7 and a relative group difference of 35%, which is a reasonable difference to expect when it is considered that recanalization rates after intra-arterial thrombolysis may be twice as high as after intravenous treatment. Our study design cannot exclude that the 2 treatments differ by a smaller degree, but all the measurements performed (perfusion-weighted imaging, ADC imaging, and ATP and pH imaging) showed that the outcome was virtually the same. The absence of any major difference is also supported by the observation that both treatments revealed the same statistically significant reduction of lesion size compared with the untreated control group.

The similar outcome in the 2 treatment groups demonstrates that in our study the local concentration of rtPA is not a limiting factor for posts ischemic reperfusion. The plasminogen binding sites at the lysis front become saturated by either application form, leading to similar efficacy of clot lysis. It is also reasonable to assume that the rheological effects are the same. In fact, as rtPA lowers blood fibrinogen content, the resulting decrease in blood viscosity would tend to improve collateral blood supply irrespective of the infarction site.

In conclusion, our data demonstrate that rtPA-induced thrombolysis reduces ischemic injury mainly by prevention of infarction growth and not by reversal of the initial ischemic impact. Further progress of thrombolytic therapy can therefore be expected only if new ways are found to delay irreversible injury in the infarct core until blood recirculation is resumed.

Acknowledgments

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