Prediction of Hemorrhagic Transformation After Thrombolytic Therapy of Clot Embolism
An MRI Investigation in Rat Brain

C. Neumann-Haefelin, PhD; G. Brinker, MD; U. Uhlenküken; F. Pillekamp, MD; K-A. Hossmann, MD, PhD; M. Hoehn, PhD

Background and Purpose—Thrombolytic treatment of stroke carries the risk of hemorrhagic transformation. Therefore, the potential of MRI for prediction of recombinant tissue plasminogen activator (rtPA)—induced bleeding is explored to identify patients in whom rtPA treatment may provoke such complications.

Methods—Spontaneously hypertensive rats (SHR) (n = 9) were submitted to middle cerebral artery (MCA) clot embolism, followed 3 hours later by intra-arterial infusion of 10 mg/kg rtPA. Untreated SHR (n = 9) were infused with saline. MRI imaging was performed before treatment and included apparent diffusion coefficient (ADC), T2, and perfusion mapping and contrast enhancement with gadolinium-DTPA. The distribution of intracerebral hemorrhages was studied 3 days later by histological staining.

Results—Clot embolism led to the rapid decline of ADC in the territory of the occluded artery. Tissue lesion volume derived from ADC imaging increased by 155±69% in the untreated animals and by 168±87% in the treated animals (P≠NS), determined on the histological sections after 3 days. This same lesion growth in both groups indicated absence of therapeutic effect after 3-hour treatment delay. Hemorrhagic transformations were significantly more frequent in treated SHR (P<0.05). In untreated rats, hemorrhages were found in the border zone of the ischemic territory; in treated animals, hemorrhagic transformations occurred in the ischemic core region. rtPA-induced hemorrhages were predicted by a disturbance of the blood-brain barrier in 3 of 4 animals before treatment by Gd-DTPA contrast enhancement but not by ADC, T2, or perfusion imaging. The region of contrast enhancement colocalized with subsequent bleeding in these animals.

Conclusions—The disturbance of blood-brain barrier but not of other MR parameters allows risk assessment for hemorrhagic transformation induced by subsequent thrombolytic treatment. (Stroke. 2002;33:1392-1398.)

Key Words: cerebral hemorrhage ■ contrast media ■ magnetic resonance imaging ■ thrombolysis ■ rats

During the past years, thrombolysis has been increasingly used for the treatment of acute ischemic stroke. Recent clinical multicenter trials documented a small but significant improvement of neurological outcome when thrombolysis was started within 3 hours after the onset of stroke symptoms.1,2 However, the increased risk of hemorrhagic transformation (HT) must be carefully weighed against the potential benefit of this treatment.3 A reliable criterion is therefore required to identify those patients in whom thrombolytic treatment might provoke such a complication.

Such a risk assessment must be made in 2 steps: first, pretreatment hemorrhages must be safely excluded to prevent likely aggravation, and then the individual risk of posttreatment hemorrhage must be determined to minimize possible complications. Pretreatment bleedings can be reliably detected by CT and susceptibility-weighted MRI. Prediction of posttreatment HT is more difficult and is therefore the subject of intense research. Clinical data suggest that bleedings may be related to disturbances of the blood-brain barrier (BBB),4 but systematic experimental studies have been precluded by the fact that HT is rare in animal models of thrombolysis. Most experimental groups have therefore looked for pretreatment signatures of cell death as potential markers of an increased risk of HT. However, basing pretreatment decisions on such signatures may lead both to unjustified inclusions and exclusions of stroke patients: unjustified inclusions because hemorrhages have been observed in undamaged brain5 and unjustified exclusions because thrombolytic protection of the penumbra may lead to the significant reduction of final lesion size, even if the infarct core is irreversibly damaged and cannot be salvaged.6,7 This raises the question of whether hemorrhages can be predicted by MRI irrespective of the severity of the ischemic injury.
Recently, we established an embolic clot model in spontaneously hypertensive rats (SHR), which exhibit a cerebrovascular pathology that resembles that of hypertensive patients followed with a high incidence of HT, particularly after intra-arterial thrombolysis with a high dose of human recombinant tissue plasminogen activator (rtPA). In the present study we used this model to investigate whether widely available MR sequences can predict HT before thrombolysis. Our findings confirm that disturbance of the BBB is, in fact, a sensitive predictor of rtPA-induced bleeding, whereas classic signatures of irreversible tissue injury are of little prognostic value.

Materials and Methods

Animal Preparation

All animal handling and surgery were performed in accordance with standard animal protection guidelines and approved by local authorities.

Eighteen male SHR were anesthetized with halothane (0.8% to 2% in 7.5 N2O/O2) under spontaneous respiration. Body temperature was kept constant at 37.5°C with the use of a feedback-controlled heating system. The tail artery was cannulated with a PE50 catheter for monitoring arterial blood pressure and sampling for blood gas analysis. Thromboembolic stroke was induced according to the method described by Busch et al. Briefly, a catheter was inserted into the right internal carotid artery, and 10 fibrin-rich autologous blood clots (each 0.35 mm in diameter and 1.5 mm in length) were injected, resulting in embolization of the right middle cerebral artery (MCA). Immediately after embolization, the catheter was retracted into the stump of the external carotid artery to avoid obstruction of the lumen of the common and internal carotid arteries.

Immediately after anesthesia, animals were positioned in the nuclear MR (NMR) magnet (see below), and a multiparametric set of MR images was acquired. Three hours after embolization, 1 group of animals (n=9) received an intracarotid injection of rtPA (10 mg/kg body wt; Actilyse, Boehringer), and a second group (n=9) received an equivalent volume of saline. Both injections were given outside the magnet after all MR measurements had been performed. At the end of the infusion, the catheter was removed, wounds were closed, and animals were allowed to recover from anesthesia.

The time interval of 3 hours between thrombolytic therapy and clot embolism was chosen on the basis of an earlier study showing that at 3 hours a positive treatment by rtPA was no longer observed. Furthermore, Brinker and colleagues showed that HTs can reliably be induced in SHR by means of the presently used animal model and timing conditions for treatment. Thus, although rtPA treatment is performed at this time point, no confounding effect by any treatment success would hide the bleeding aspects of our study.

For histological staining, animals were reanesthetized at day 3 after embolization and transcardially fixed with a 3% formalin solution for 3 days and frozen. Coronal cryostat sections (10 μm in thickness) of forebrain, prepared at the levels of MR slices, were stained with cresyl violet and hematoxylin-eosin for visualization of hemorrhages. Frozen brains from animals that died before day 3 (n=4 in the control group and n=3 in the treated group) were investigated histologically in the same way. Animals that showed no ischemic signal changes in either perfusion-weighted images or apparent diffusion coefficient (ADC) maps (n=2) were excluded from further investigations, leading to n=7 in the treated group and n=9 in the control group.

MRI Measurements

Multiparametric MR measurements were performed 2 to 3 hours after embolism with a 4.7-T MSL-X11 BIOSPEC experimental scanner (Bruker Medical GmbH) with a 30-cm-bore magnet and actively shielded gradient coils (100 mT/m; rise time <250 μs). A 12-cm

Helmholtz coil was used for radiofrequency transmission; signals were detected with a 16-mm inductively coupled surface coil placed over the skull of the animal. The radiofrequency coils were decoupled from each other (the transmitter coil actively and the receiver coil passively).

With the use of gradient echo imaging, sagittal pilot scans were performed to ensure accurate positioning of the animal in the magnet. For this purpose, the coronal plane 5.9 mm posterior to the rhinal fissure was placed in the isocenter of the magnet, thus focusing on the center of the ischemic territory in the MCA occlusion model.

For a detailed comparison with histological data, different NMR imaging modalities were used. Unless described otherwise, a field of view of 4.0 cm, a matrix of 128×128, a slice thickness of 1.21 mm, and an interslice distance of 1.75 mm were used for all sequences. Six coronal slices were recorded, placing the center of the multislice imaging packet 6.7 mm posterior to the rhinal fissure.

Diffusion-weighted imaging was performed with the use of a Stejskal-Tanner spin-echo sequence (echo time [TE]=35.2 ms; repetition time [TR]=23.25 ms). To enable quantification of the ADC of brain water, 3 b values were used (b=30, 765, and 1500 s/mm²). ADC maps were calculated pixelwise with the use of the MEMRIS software package, written in Interactive Data Language (IDL, Research Systems Inc).

Coronal multislice T2-weighted imaging was performed with a multislice, multiecho Carr-Purcell-Meiboom-Gill (CPMG) sequence (TE=13.1 ms; 8 echoes; TR=3000 ms) at the same 6 levels as the diffusion-weighted imaging experiment. Sixteen echoes were collected for calculation of brain water T2 maps.

Perfusion-weighted imaging was performed with an ultrafast version of the arterial spin-tagging technique. One coronal slice, placed in the isocenter of the magnet, was recorded, thus covering the central part of the ischemic lesion. Measurement parameters were as follows: TE=3.5 ms; TR=7.4 ms; slice thickness=2 mm; matrix=128×64; averages=8; total scan time=1.3 minutes. The sequence consisted of 2 image acquisitions separated by a recovery period. During the first acquisition, blood flowing through the neck was adiabatically inverted; in the second acquisition, inflowing spins were left undisturbed. Perfusion-weighted images were obtained by subtraction of the acquisitions with and without prior adiabatic spin inversion.

The permeability of the BBB was assessed at the end of the MR investigation with the use of gadolinium-DTPA (Gd-DTPA) (0.2 mL IV; Schering). Gd-DTPA–enhanced images were obtained with the use of a T1-weighted imaging sequence (TE=8.3 ms; TR=400 ms). Contrast enhancement was visualized by subtraction of the precontrast images from images obtained 5 minutes after the injection of Gd-DTPA. To determine areas with BBB disturbance, we included all pixels with a signal intensity increased by 2 SD above the signal intensity of the contralateral hemisphere.

Data Analysis and Statistical Evaluation

Statistical differences between treated and untreated groups in respect to the incidence of hemorrhages were calculated with the Mann-Whitney U test after grading for hemorrhages as follows: 0, no hemorrhage; 1, small area of bleeding in a single location identified microscopically but not macroscopically (single perivascular diapedesis); 2, same as 1, but in multiple areas (multiple diapedesis); 3, macroscopically detectable non–space-occupying hemorrhage; and 4, macroscopically detectable space-occupying hemorrhage.

MR images were transferred to a Macintosh Power PC 7200/66 (Apple). For further investigations, 3 regions of interest (ROIs) were selected with the use of the NIH Image Program (National Institutes of Health), as follows: ROI 1, that part of the ischemic territory in which ADC had declined to <50% of the contralateral value before rtPA treatment; ROI 2, regions showing Gd-DTPA enhancement before treatment; and ROI 3, regions showing HT in histological sections obtained after 3 days. Those ROIs of HT (as identified both macroscopically and microscopically) were first delineated on schematic sections at levels corresponding to the stained histological sections. Subsequently, a different observer transferred these ROIs to

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Pretreatment Recordings of ADC, T2, and Signal Intensities of Perfusion-Weighted Images and Gd-DTPA-Enhanced T1-Weighted Images in Ischemic Areas With and Without HT at 3 Days After Thromboembolism

<table>
<thead>
<tr>
<th></th>
<th>Untreated Group</th>
<th>rTPA-Treated Group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HT Area</td>
<td>Non-HT Area</td>
</tr>
<tr>
<td>ADC</td>
<td>0.76±0.08</td>
<td>0.63±0.06</td>
</tr>
<tr>
<td>T2</td>
<td>1.13±0.06</td>
<td>1.18±0.04</td>
</tr>
<tr>
<td>Perfusion-weighted imaging</td>
<td>0.40±0.20</td>
<td>0.43±0.17</td>
</tr>
<tr>
<td>Gd-DTPA</td>
<td>1.02±0.04</td>
<td>1.00±0.03</td>
</tr>
</tbody>
</table>

Values refer to the homologous contralateral regions and are given as ratios of ipsilateral to contralateral regions.

*P<0.05. Differences were significant only for Gd-DTPA enhancement in the rtPA-treated group.

Results

All physiological parameters were kept within the normal range during MR measurements and did not differ between groups.

NMR Measurements Before Treatment

Clot embolism produced a decline of ADC in the territory of the occluded artery in all included animals. At 2.5 hours after embolism, the hemisphere lesion volume with ADC values <80% of the contralateral hemisphere was 35.6±22.8% in animals selected for treatment and 31.1±15.1% in the untreated animals (P=NS). Three days after embolism, infarct volume measured by histology was 42.8±12.4% and 48.3±22.4% in treated and untreated animals, respectively (P=NS). Lesion growth during the initial 3 days of infarction was very similar between groups and amounted to 155.2±68.7% in the treated animals and 167.5±87.4% in the untreated animals. Seven animals died within 24 hours, 4 in the treated group and 3 in the control group. Premature death occurred in the 3-day survival group at 4, 5, and 6 hours after embolization among treated animals and at 8 and 24 hours among untreated animals. These animals showed significantly larger hemispheric lesion volumes (on ADC maps) than animals that had survived for 3 days (P<0.001) but showed no difference in the development of HT. Distinctly more pronounced edema was not noted in these prematurely dead animals.

Measurements of T2 and perfusion signal intensities before thrombolysis showed no difference in the mean values between those ischemic areas that did and those that did not undergo subsequent HT (Table). The observed values were typical for the time point chosen, ie, 2.5 to 3 hours after MCA occlusion. Only the comparison of the ADC within the region of later bleeding showed a modestly but not significantly higher value in animals without treatment than in treated animals (0.76±0.08 and 0.66±0.06, respectively).

Ventricular contrast enhancement after injection of Gd-DTPA was found in all animals before therapy was started. However, parenchymal contrast enhancement (due to BBB damage) was found only in a subset of animals in the treated (n=5/7) and the untreated (n=5/9) group. In the remaining animals, contrast enhancement was restricted to the ipsilateral ventricle (n=4 and n=2 in control and rtPA-treated animals, respectively). There was no difference in the size or the location of the area showing Gd-DTPA enhancement between treated and untreated animals (before treatment).

Incidence of HT After Treatment

Histological examination revealed a significantly higher incidence of hemorrhages in rtPA-treated animals than in the untreated group (Figure 1). In the untreated group, 5 of 9 animals exhibited a diapedetic hemorrhage (histological score 2), 1 animal showed a single perivascular diapedesis of blood (histological score 1), and 3 animals developed no HT. In contrast, rtPA treatment resulted in diapedetic or macroscopically visible bleedings (score 2 or 3) in 5 of 7 animals (P<0.05). Typical examples of hemorrhages are shown in Figure 2.

In addition to the differences in bleeding severity, areas with HT were located in different subregions of the infarcts. In animals that did not receive rtPA, HT was found only in the periphery of the ischemic lesions, whereas in treated animals bleedings were detected mostly in the center of the ischemic territory. This is reflected by the distribution of ADC values in areas showing subsequent hemorrhages (Fig-
ure 3). In the untreated group, HT was mainly found in regions with only moderately reduced ADC (80% to 100%), which corresponds to the ischemic penumbra, whereas ADC in HT regions of treated animals declined below this value.

**Prediction of HT**

As a potential marker of subsequent HT, BBB damage before treatment was further investigated. Pretreatment parenchymal BBB disturbances were found in 10 animals (n=5 in each group). Typical examples of BBB disturbance and the ischemic territory are given in Figure 4. We found a close correlation between Gd-DTPA–induced contrast enhancement due to BBB disturbance and the observation of subsequent HT in rtPA-treated animals. On the other hand, BBB disturbance as registered by signal enhancement after Gd-DTPA injection was not correlated with HT in untreated animals.

In more detail, in the rtPA-treated group, animals with a BBB disturbance showed significantly larger HT (Figure 5; histological score, 2.8) than animals without BBB disturbance. Furthermore, in all rtPA-treated animals with BBB disturbance, the area of pretreatment BBB damage closely matched the area of later HT (Figure 6).

**Discussion**

Intracerebral hemorrhage with clinical deterioration represents the most important complication and safety issue in the use of thrombolytic agents in patients with acute stroke. Until now, only a few studies using animal models of acute cerebral ischemia have addressed the incidence and mechanisms of intracerebral hemorrhage after thrombolytic therapy. In this study we investigated whether the risk of HT can be predicted by modern MRI methods.

With respect to anatomic structures and the distribution of ADC values, we could demonstrate that hemorrhages in untreated animals were located mainly in the periphery of the infarct, while bleeding in rtPA-treated animals was distributed randomly in the infarcted area. Bleedings were never found in nonischemic ipsilateral regions or in the contralateral hemisphere. This confirms our previous observation that even in the presence of severe cerebrovascular disease, thrombolysis does not increase the risk of HT in nonischemic tissue of SHR.

**Prediction of HT Using ADC Maps**

In a human study, Tong et al described the possibility of distinguishing between areas showing subsequent HT and non-HT ischemic regions using a pixel analysis on ADC maps under ischemic conditions. Regions that experienced HT exhibited a higher percentage of ischemic tissue in a low ADC range than non-HT–destined lesions. In contrast, the mean ADC value in areas with subsequent HT was not significantly different from that of ischemic regions without later bleedings. These results are in agreement with our data in the treated group, in which no significant difference between the ADC in regions with and without later bleedings could be found. In fact, in untreated animals mean ADC was even higher in areas with subsequent HT than in non-HT areas. The ADC in HT regions of treated rats showed typical ischemic values. This difference between both groups may be related to the particular subtype of HT observed in the untreated group. HT in these animals consisted mainly of
small bleedings, which appear to be common in human stroke but which are frequently not associated with neurological deterioration.  

**Prediction of HT Using Gd-DTPA Contrast Enhancement**

We found a close correlation between HT and pretreatment BBB damage as detected by post–contrast enhanced T1-weighted imaging. Animals showing contrast enhancement due to a disturbance of the BBB developed significantly greater and more severe HT after rtPA injection than animals without BBB disturbances. Only animals with BBB damage showed macroscopically detectable bleedings (score 3), similar to symptomatic HT in human stroke associated with neurological deterioration. Therefore, the main finding of our study is that areas showing a BBB disturbance before thrombolysis are at considerably increased risk for HT after treatment.

The reason for the increased risk of HT after thrombolysis is not completely understood. Several factors have been discussed, however. Time to treatment, hypertension, and age were found to be risk factors for the development of parenchymal hemorrhages. Hamann et al showed that disturbances of the BBB are detectable at early time points after an ischemic event and increase in severity with increasing time of vessel occlusion. Early endothelial ischemic damage is followed by the loss of microvascular permeability barriers. This is in full agreement with our present study: small molecules (ie, Gd-DTPA) can extravasate and become visible in contrast-enhanced MR images. In our study 10 of 16 animals showed a BBB disturbance after injection of Gd-DTPA between 2 and 3 hours after embolization.

In the present study we have shown that more severe hemorrhagic complications occur in SHR after thrombolytic treatment than without rtPA treatment. This is in full agreement with clinical trials. However, only a few experimental studies have reported similar findings, possibly because of the rare occurrence of HT in animal models. Zhang et al and del Zoppo et al did not observe a significant difference
between untreated and rtPA-treated animals when treatment was initiated at early time points after embolism. Overgaard et al\(^2\) reported absence of confluent hemorrhages in rats even after a treatment delay of 4 hours. We expect that this discrepancy between clinical and animal studies is due to the fact that most experimental models of focal ischemia do not mimic the age-dependent vascular changes. However, the model used in the present study (in particular the use of SHR) probably resembles the clinical situation much better and offers the opportunity to investigate the pathophysiology of postthrombotic hemorrhage.

There have been several previous reports of clinical and experimental stroke studies using Gd-DTPA as BBB tracer in the acute phase of cerebral ischemia.\(^2,4,25\) Nevertheless, studies that combine this method of contrast-enhanced MRI with the evaluation of the risk of hemorrhagic complications are rare. Yenari et al\(^2\) demonstrated in a rabbit model that contrast-enhanced scans can reveal regions of BBB disruption in the presence of HT. Using T1-weighted imaging after administration of Gd-DTPA, Knight et al\(^2\) found a close correlation between Gd-DTPA enhancement after reperfusion and the development of HT in rats. In a human case report, Gd-DTPA contrast enhancement in T1-weighted images also correlated with HT, determined by a CT scan at a later time point.\(^2\) However, these findings do not necessarily indicate the possibility of detecting tissue at risk of HT before bleeding occurs or before reperfusion is initiated.

However, a close correlation between rtPA-induced HT and BBB breakdown was found in an experimental study reported by Dijkhuizen et al.\(^2\) Despite the later rtPA administration time point (6 hours), the model used by those authors was very similar to the model described in the present study, and the authors arrived at nearly identical results when analyzing the correlation between BBB damage and HT. However, using perfusion-weighted imaging, Dijkhuizen et al found significantly lower regional cerebral blood flow values in regions of subsequent bleedings, a result that could not be found in the present study. Finally, our ADC analysis is more detailed because a complex pixel analysis was performed to describe the ADC values in regions developing later HT in the different treatment groups, separate from those pixels of the ischemic territory without HT.

In contrast, Neumann-Haefelin et al\(^3\) found that early BBB damage after reperfusion was not associated with subsequent HT after transient MCA occlusion in normotensive untreated rats. This result is in accord with our untreated group, in which HT consisted mainly of small hemorrhages and in which BBB disturbances and HTs did not correlate. However, that result seems to be completely different after rtPA treatment, when the increased risk of HT can be detected with the use of Gd-DTPA–enhanced T1-weighted imaging.

### Conclusions

ADC, T2, or perfusion mapping clearly demarcates the ischemic area from the nonischemic regions, but these parameters do not predict subsequent HT. HT prediction is possible, however, with the use of contrast-enhanced MRI. In the rtPA-treated group we found a good correlation between pretreatment BBB damage and subsequent HT severity, demonstrating that BBB damage heralds HT before thrombolytic intervention is initiated. Pretreatment BBB damage, as detected by contrast-enhanced T1-weighted imaging, may therefore become an important parameter for rtPA treatment selection in the future.

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