Serial MRI After Transient Focal Cerebral Ischemia in Rats
Dynamics of Tissue Injury, Blood-Brain Barrier Damage, and Edema Formation

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Background and Purpose—With the advent of thrombolytic therapy for acute stroke, reperfusion-associated mechanisms of tissue injury have assumed greater importance. In this experimental study, we used several MRI techniques to monitor the dynamics of secondary ischemic damage, blood-brain barrier (BBB) disturbances, and the development of vasogenic edema during the reperfusion phase after focal cerebral ischemia in rats.

Methods—Nineteen Sprague-Dawley rats were subjected to transient middle cerebral artery occlusion of 30 minutes, 60 minutes, or 2.5 hours with the suture occlusion model. MRI, including diffusion-weighted imaging (DWI), T2-weighted imaging, perfusion-weighted imaging, and T1-weighted imaging, was performed 5 to 15 minutes before reperfusion, as well as 0.5, 1.5, and 2.5 hours and 1, 2, and 7 days after withdrawal of the suture. Final infarct size was determined histologically at 7 days.

Results—In the 30-minute ischemia group (and partially also after 60 minutes), DWI abnormalities reversed transiently during the early reperfusion period but recurred after 1 day, probably due to secondary ischemic damage. After 2.5 hours of ischemia, DWI abnormalities no longer reversed, and signal intensity on both DWI and T2-weighted images increased rapidly in the previously ischemic region due to BBB damage (enhancement on postcontrast T1-weighted images) and edema formation. Early BBB damage during reperfusion was found to be predictive of relatively pronounced edema at subacute time points and was probably related to the increased mortality rates in this experimental group (3 of 7).

Conclusions—Reperfusion after short periods of ischemia (30 to 60 minutes) appears to be mainly complicated by secondary ischemic damage as shown by the delayed recurrence of the DWI lesions, whereas BBB damage associated with vasogenic edema becomes a dominant factor with longer occlusion times (2.5 hours). (Stroke. 2000;31:1965-1973.)

Key Words: blood-brain barrier □ brain edema □ cerebral ischemia, focal □ magnetic resonance imaging □ reperfusion □ rats

MRI techniques, including diffusion-weighted imaging (DWI) and perfusion-weighted imaging (PWI), have been used increasingly in recent years to evaluate various treatment modalities in both experimental stroke models1–6 and acute stroke patients.7,8 In human stroke, only agents that lead to a reopening of the occluded artery (ie, to reperfusion, before ischemic tissue injury is complete) have proved to be beneficial so far.9,10 However, even with thrombolytic treatment, which is the current treatment standard for selected patients within 3 hours of stroke onset, overall patient benefit remains less than optimal. This is in part due to an increase in the risk of symptomatic intracranial hemorrhage among patients treated with recombinant tissue plasminogen activator (rtPA).11 However, various reperfusion-induced processes (“reperfusion injury”) are believed to play an important role as well, and they have been investigated extensively in experimental studies.12,13 Previous reports have implicated secondary hemodynamic disturbances,14,15 the enhancement of inflammatory processes,16,17 free radical formation,18 vasogenic edema, and breakdown of the blood-brain barrier (BBB)19 as factors that contribute to reperfusion injury. The potential to monitor the development of secondary or delayed ischemic damage with MRI was the focus of several recent reports.20–23 Earlier studies that used DWI to investigate the effects of reperfusion reported a reversal of DWI abnormalities during reperfusion after focal ischemia of up to 60 minutes.24–27 Recent studies, however, indicate, that the reversal of acute DWI abnormalities may be only transient and that ischemic tissue injury may become detectable again at later time points, both with MRI and histologically.20,22,23,28 The delayed recurrence of the DWI lesions after brief

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ischemic episodes indicates that secondary damage may occur in ischemic regions despite an acute reversal of DWI abnormalities.

The development of vasogenic edema due to BBB disturbances after reperfusion and its influence on ischemic lesion evolution has received comparatively little attention in experimental MRI studies, and data are available only for the acute reperfusion phase.\(^{29,30}\) On the other hand, numerous histological studies with various tracers have established that recirculation may exacerbate vasogenic edema due to BBB disruption.\(^ {12,19,31,32}\) Vasogenic edema is well recognized as a major factor that contributes to early death in patients with relatively large strokes. In addition, BBB breakdown has been associated with an increased risk of hemorrhagic transformation.\(^ {33}\) The main advantage of MRI over histological methods is that it is noninvasive and thus can be used to monitor the evolution of BBB breakdown and the development of vasogenic edema over time in the same experimental subjects.

The aim of the present serial MRI study was to investigate the temporal evolution of reperfusion-associated injury (secondary ischemic damage, BBB breakdown, and vasogenic edema) during 1 week with a model of reversible focal cerebral ischemia. We chose to investigate 3 experimental groups with potentially clinically relevant ischemia durations (0.5, 1, and 2.5 hours). An MRI protocol consisting of DWI, T2-weighted imaging (T2WI), PWI, and postcontrast T1-weighted imaging (T1WI) was performed during ischemia and at 6 time points during the subsequent 7 days.

**Materials and Methods**

**Animal Model and Protocol**

The study was approved by the institutional review board of our institution. Focal cerebral ischemia was introduced in 19 male Sprague-Dawley rats (300 to 340 g) with the intraluminal suture occlusion model.\(^ {34}\) Briefly, animals were spontaneously breathing and anesthesia was induced with 3% halothane in a mixture of air and anesthesia was induced with 3% halothane in a mixture of air and was further reduced to 0.75% to 1% during imaging. Body temperature was maintained at 37 ± 1°C with a warm air circulation system and measured with a rectal probe. Pulse oximetry was used to monitor oxygen saturation and heart rate. The femoral vein was catheterized to deliver the contrast agent.

Middle cerebral artery (MCA) occlusion (MCAO) was achieved by introducing a 4-0 silicone-coated filament into the distal common carotid artery, which was then advanced via the internal carotid artery until a faint resistance was felt (~20 mm from the carotid bifurcation). Immediately after the occlusion, the animals were positioned in a plastic headholder with ear bars and transferred to the MRI scanner. For withdrawal of the sutures, the animals were removed from the magnet for ~5 to 10 minutes and then carefully repositioned (using EPI scout imaging and subsequent corrections, if necessary) for an additional 2.5 hours of imaging, before they were returned to their cages with free access to food and water. At 1, 2, and 7 days after MCAO, the animals were reanesthetized for additional MRI.

The study protocol consisted of 3 groups with either 30 minutes (n = 5), 1 hour (n = 7), or 2.5 hours (n = 7) of MCAO with 7-day survival. MRI was performed 5 to 15 minutes before reperfusion and 0.5, 1.5, and 2.5 hours and 1, 2, and 7 days after withdrawal of the suture.

**MRI**

The MRI experiments were performed with a 2.0-T GE CSI system (Bruker Instruments) equipped with shielded gradients capable of producing 20 G/cm. The rat was positioned supine with the head inside a 5.5-cm-diameter bird cage radiofrequency coil. To monitor infarct evolution, diffusion- and T2-weighted images were acquired at each imaging time point (see earlier). In addition, PWI was performed at all time points up to 2.5 hours after withdrawal of the suture to determine adequate reperfusion. At ~10 minutes after each application of contrast agent for PWI, T1-weighted images were acquired to assess BBB damage.

For DWI, an isotropic diffusion-weighted spin-echo sequence was used as described previously.\(^ {35}\) Briefly, each set of diffusion-sensitizing gradients before and after the radiofrequency pulse had a duration of 25 ms. We used b values of 1300 and 20 s/mm\(^2\) for DWI and T2WI, respectively. From these images, pixel-by-pixel maps of the apparent diffusion coefficient (ADC) of water were calculated. The images were acquired with a 128 × 128 matrix, field of view of 50 mm, repetition time (TR) of 2.5 s, echo time (TE) of 80 ms, 1 average, 8 coronal slices, slice thickness 1.5 mm, and interslice gap of 0.2 mm.

Bolus-tracking PWI was performed with spin-echo echo planar imaging (EPI) with the following parameters: TE 88 ms, TR 1 s, 1 average, 2 coronal slices, slice thickness 1.5 mm, and interslice gap 0.2 mm. Forty images were acquired during 40 s for 2 central slices. A bolus of 0.3 mmol/kg gadolinium-DTPA (Magnevist; Schering) was injected after the first 10 baseline scans.

For T1WI, the following parameters were used: matrix 128 × 128, field of view 50 mm, TE 14 ms, TR 300 ms, 2 averages, 8 coronal slices, slice thickness 1.5 mm, and gap 0.2 mm.

**Histology**

After completion of the imaging protocol at 7 days, animals were transcardially perfused with fixative containing 4% paraformaldehyde in PBS for histology. The brains were cryoprotected with the same fixative containing 20% sucrose. Sections (20 μm) were cut on a cryostat at −20°C and stained with cresyl violet. Infarcts were outlined on the central section of each animal by a blinded investigator, and corrected hemispheric lesion areas (in percentages; see Data Analysis for details) were determined with a computer-assisted image analysis system (MCID).

**Data Analysis**

To assess the evolution of ischemic injury during ischemia and after reperfusion, we calculated the changes in lesion volume and signal intensity on DWI, T2WI, and ADC maps. Perfusion-weighted images were assessed only qualitatively, both with the raw data and with calculated time-to-peak maps, to determine adequate reperfusion. Successful reperfusion was assumed if PWI abnormalities either completely or almost completely normalized after withdrawal of the sutures. DWI and T2WI lesion volumes were determined by a blinded investigator experienced in experimental stroke MRI with the image processing software MRVision (MRVision Co). After optimal adjustment of contrast, the edge of the lesions was traced manually on each of the 8 coronal slices, which completely covered the middle cerebral artery territory in all animals. The areas of hyperintensity were then summed and multiplied by the slice thickness plus interslice gap to calculate lesion volumes. A subset of data were analyzed by a second observer to determine the interobserver reliability (correlation coefficient r = 0.92). To facilitate the comparison between animals, we normalized the lesion volumes to the last DWI lesion volume obtained during ischemia (for the 30-minute ischemia group, ~25 minutes postocclusion; 60-minute ischemia group, ~50 minutes postocclusion; 2.5-hour ischemia group, ~130 minutes).

For comparison with histology, the corrected hemispheric lesion areas (HLAs) were calculated with the equation\(^ {36}\) HLA (mm\(^2\)) = [LT − (RT − RI)]/LT × 100, where LT is the area of the left hemisphere (in mm\(^2\)), RT is the area of the right hemisphere (in mm\(^2\)), and RI is the infarcted area (in mm\(^2\)). With this correction.
procedure, the effects of tissue shrinkage (due to fixation) as well as edema formation on the estimation of infarct size may be minimized.

For the monitoring of signal intensity changes, a region of interest (ROI) was defined on the central DW image obtained during ischemia that completely covered the hyperintense region. This ROI was then transferred to both the corresponding DW images obtained during reperfusion as well as the T2WI and the ADC maps. Control values for each of the parameters (DWI, T2WI, ADC) were determined from an ROI drawn in unaffected tissue contralaterally (same anatomic structures as ipsilaterally), and all values of signal intensities given in this report are hemispheric ratios (ratio of ipsilateral to contralateral signal intensities).

Statistical analysis was performed with 1-factor ANOVA followed by a post hoc analysis with Bonferroni’s correction when testing for differences between the 3 experimental groups. When testing for changes in single parameters in individual groups over time, 1-factor ANOVA was used, followed by a post hoc analysis with Dunnett’s procedure. With Dunnett’s procedure, we made multiple comparisons of postrecirculation values against the last intracranial value obtained before reperfusion. Statistical significance was inferred at P<0.05. Data are presented as mean±SD (except for Figures 2 and 3, where error bars indicate the SEM).

Results
In all animals included in the study, PWI confirmed successful reperfusion after withdrawal of the suture. One animal (1 of 7) in the 1-hour ischemia group died between 24 and 48 hours after reperfusion, and 3 rats (3 of 7) in the 2.5-hour ischemia group died between 2.5 and 24 hours after reperfusion. All rats in the 30-minute ischemia group survived to the last imaging time point (7 days).

Changes in ADC Values and Signal Intensities on Diffusion- and T2-Weighted Images After Reperfusion
Figure 1 illustrates the characteristic MRI patterns observed during reperfusion after 30 minutes and 2.5 hours of ischemia in 2 representative animals. Figure 2 depicts the average changes in signal intensity on ADC maps, DWI, and T2WI.

Before reperfusion, average ADC values and signal intensities on diffusion- and T2-weighted images of the ischemic regions did not differ significantly between the 0.5- and 1-hour ischemia groups. Signal intensity was, however, significantly greater on both diffusion- and T2-weighted images (P<0.01) and ADC values were lower (P<0.05) in the 2.5-hour ischemia group than in the 0.5- and 1-hour groups.

After the initiation of reperfusion, the appearance of the ischemic lesions changed substantially and differed among the 3 subgroups (Figure 2). In the 0.5-hour ischemia group,
the average ADC value of the ischemic regions recovered rapidly and almost completely (from 71\% to 90\% during ischemia to 90\% at 2.5 hours after reperfusion). When excluding the only animal in this group (1 of 5) with no relevant ADC change after reperfusion, ADC returned to 98\% at 2.5 hours after reperfusion. However, at 1 day after reperfusion, ADC values had again declined to 71\%, before returning to (pseudonormal) values at 2 and 7 days. The initial recovery and the secondary decline of ADC values were associated with an almost complete normalization of intraischemic DWI abnormalities during early reperfusion, followed by a secondary increase in signal intensity at 1 day. On T2-weighted images, the most pronounced increase in signal intensity was found at 1 day (35\%), but there also was a minor nonsignificant increase during the first 2.5 hours of reperfusion (by 8\%). Signal intensity on T2-weighted images dropped again substantially between 2 and 7 days (to 12% above control).

In the 2.5-hour ischemia group, in contrast, ADC remained low during the early reperfusion period (<2.5 hours) but started to increase at 1 day and normalized between 2 and 7 days. Although ADC changed little during the early reperfusion period in this group, signal intensity increased sharply on diffusion- and T2-weighted images almost immediately after reperfusion. Signal intensity reached peak values by 2.5 hours and 1 day, before partially declining again at 2 and 7 days.

The changes in the 1-hour ischemia group were similar to those found in the 0.5-hour ischemia group, but the initial recovery of ADC values during early reperfusion was not as uniform as in the 0.5-hour group. Typically, ADC recovery was more pronounced in cortex than in the caudoputamen. In 2 animals (2 of 7), the average ADC of the ischemic region remained low or even decreased after reperfusion; as in the 2.5-hour ischemia group; this was associated with a rapid increase in T2 signal intensity.

**Apparent Lesion Volumes**

Lesion volumes (Figure 3) were determined on diffusion- and T2-weighted images during ischemia (just before reperfusion) and at all time points after reperfusion. There was no statistically significant difference between DWI lesion volumes in the 3 experimental groups before reperfusion. No lesions could be identified on T2-weighted images before reperfusion.

In the 0.5-hour ischemia group, the apparent DWI lesion volumes decreased on average by 90\% (P<0.01) during the first 2.5 hours of reperfusion. In 3 of 5 rats of this group, lesions were no longer detectable during early reperfusion. At 1 and 2 days, however, mean DWI lesion size had increased again to 78\% and 80\% of the preperfusion size, respectively. On T2-weighted images, lesions in the 0.5-hour group were first detected at 1 day after reperfusion and lesion volumes peaked at 2 days, at which point they were similar to...
the intraischemic lesion volumes and slightly larger than the current DWI lesion volumes.

The time course of lesion evolution in the 1-hour ischemia group was similar to that of the 0.5-hour group, but DWI lesion volumes decreased by only 62±36% (P<0.05) during early reperfusion.

In the 2.5-hour ischemia group, DWI lesion volumes did not change at all after reperfusion (up to 2 days). In contrast to the other 2 groups, lesions became rapidly visible on T2-weighted images after reperfusion and peak volumes were substantially larger than the intraischemic DWI abnormalities (208%). In all 3 groups, apparent lesion volumes on T2-weighted images decreased substantially between 2 and 7 days (to about one third of peak lesion volumes at 2 days).

Histologically, infarcts were seen in all animals, even in those with complete reversal of the DWI lesions after reperfusion. The mean histological HLA (%) was not significantly different among the 3 experimental groups (36±14%, 33±21%, and 27±24% for the 30-, 60-, and 150-minute MCAO groups, respectively). For all groups, histological HLA was slightly smaller (NS) than the intraischemic (pre-reperfusion) DWI HLA (41±14%, 53±18%, and 33±4% for the 30-, 60-, and 150-minute MCAO groups, respectively). Surprisingly, in both the 30- and 60-minute ischemia groups, lesions consistently appeared smaller on T2-weighted images obtained at 7 days than histologically but not in the 2.5-hour group (T2WI HLA at 7 days: 11±20%, 3±11%, and 33±11% for the 30-, 60-, and 150-minute MCAO groups, respectively).

**Discussion**

In this serial MRI study, we delineated the dynamics of several reperfusion-associated processes, including the delayed recurrence of DWI abnormalities, BBB damage, and vasogenic edema formation after 30, 60, and 150 minutes of MCAO, in the rat suture model. Our results confirm the important finding of a delayed recurrence of DWI abnormalities after 30 to 60 minutes of ischemia, which may occur due to secondary ischemic injury. In addition, the results extend previously existing data by showing that reperfusion-associated BBB damage occurs only with occlusion times of at least 60 minutes. More importantly, early BBB damage after reperfusion, which consistently occurs after 2.5 hours, appears to be predictive of relatively pronounced transient increases in apparent T2 lesion size due to vasogenic edema at subacute time points (1 to 2 days).

The delayed recurrence of DWI lesions at 12 to 24 hours after 30 minutes of ischemia was recently found by several investigators using either an intraluminal suture model, temporary ligation of the intracranial MCA, or an hypoxia-ischemia model. Similar work established that tissue damage may be detected histologically in ischemic regions (at 72 hours) despite an acute reversal of DWI abnormalities. In earlier studies, the acute reversal of DWI abnormalities after up to 60 minutes of ischemia was also observed, but the delayed recurrence of the DWI lesions was not recognized, because imaging was not performed at time points later than 7 hours. In 2 of these studies, TTC staining at 24 hours was used to determine final infarct size. In both studies, DWI lesions partially reversed after either 45 minutes or 1 hour of MCAO, and the size of the DWI...
abnormality during reperfusion was reported to accurately predict final infarct size.25 Our study, in contrast, suggests that the partial reversal of DWI lesions seen during early reperfusion after 1 hour of ischemia is only temporary and does not predict a favorable long-term outcome. The most likely explanation for this discrepancy is that TTC staining at 24 hours underestimated final infarct size, because infarct evolution has been shown to be relatively slow after brief focal ischemia and can take several days.38,39

From a pathophysiological point of view, the delayed recurrence of DWI lesions after brief focal ischemia is suggestive of secondary or delayed ischemic damage. A variety of different mechanisms could contribute to secondary ischemic tissue injury, including hemodynamic and metabolic disturbances (mitochondrial impairment,40 persistent inhibition of protein synthesis,41 the formation of free radicals18 ) and apoptosis. However, the reversal of MRI abnormalities during the early reperfusion period is not in itself conclusive evidence that the previously ischemic tissue is viable. Theoretically, DWI could be insensitive to ischemic tissue damage during early reperfusion, but a transient metabolic recovery of the previously ischemic region appears to be the more likely cause for the reversal of the DWI abnormalities. Evidence for a transient metabolic recovery was recently obtained in a neonatal hypoxia-ischemia model with MR spectroscopy,43 but whether this is also the case in the adult rat brain remains to be determined.

After 2.5 hours of MCAO, the most impressive changes after reperfusion were found on T2-weighted and postcontrast T1-weighted images. The rapid increase in signal intensity on T2-weighted images is highly suggestive of a net increase in mobile water content in the previously ischemic region, whereas the enhancement on postcontrast T1-weighted images (which was most pronounced in the core region) indicated BBB breakdown.29 Although apparent lesion size did not change on diffusion-weighted images during the early reperfusion period, signal intensity on DWI increased considerably despite unchanged ADC values. Because both ADC and T2 influence signal intensity on DWI, this increase in signal intensity on DWI was probably due to an increase in the T2 contribution to the DWI signal.

Early breakdown of the BBB during recirculation after long occlusion times (2 to 3 hours) has also been found in a previous MRI study29 and other studies that used tracer techniques to measure BBB damage.19,32 but long-term follow-up with MRI has not been reported. Our results show that apparent lesion size as measured on T2-weighted images increases further in the subacute period, peaking at ~48 hours after 2.5 hours of ischemia, and may considerably exceed the size of the intraischemic DWI abnormalities. Most of this secondary increase in apparent lesion size in the 2.5-hour group was probably due to vasogenic edema (rather than to the recruitment of additional tissue into the infarct), because final infarct size (as measured with histology at 7 days) did not exceed the intraischemic DWI lesion size. Most likely, vasogenic edema was also the main cause for the high mortality rate (3 of 7) in the 2.5-hour ischemia group compared with the 2 other experimental groups (30 minutes ischemia, none of 5; 60 minutes of ischemia, 1 of 7). Baseline DWI lesion volumes before reperfusion were not significantly different among the 3 groups, indicating that the excess mortality rate was not simply due to a larger area of prereperfusion damage. Taken together, our results suggest that an early increase in T2 signal intensity during the first hours of reperfusion is predictive of relatively severe vasogenic edema at subacute time points, which in turn may lead to fatal increases in intracranial pressure with large infarcts.

Between 2 and 7 days after reperfusion, apparent lesion size as measured with DWI and T2WI decreased considerably in all experimental groups. Similar decreases in apparent lesion volumes have been reported in a serial MRI study that investigated lesion evolution in human stroke.44 The decline in apparent lesion size on DWI was not surprising because ADC values are known to increase after ~24 hours and to reach “pseudonormal” levels by 2 to 7 days. The magnitude of the decrease in apparent lesion size on T2-weighted images, on the other hand, was greater than anticipated. Particularly with short occlusion times, the average apparent lesion size on T2-weighted images at 7 days was strikingly smaller than the histologically determined infarct size at the same time point. In fact, in most animals the lesions became so inconspicuous on T2-weighted images at 7 days that it became difficult to reliably define a lesion. The decrease in apparent lesion volume and lesion conspicuity on T2-weighted images between 2 and 7 days may be explained in part by the resolution of vasogenic edema, but this does not explain why lesions would appear smaller on T2-weighted images than histologically. A transient decrease in T2 lesion conspicuity between 1 and 2 weeks after stroke has also been found in a few case studies of human stroke,45,46 and the term “MR-fogging” (in analogy to the “fogging effect” frequently seen on CT) has been coined. The underlying mechanisms have not been studied systematically; factors that could contribute to a reduced lesion conspicuity on T2-weighted images include the invasion of inflammatory cells into the infarct, an increase in the number of glial cells (gliosis), or petechial hemorrhages.

Several observations of our study may be of potential clinical relevance. First, the reversal of acute DWI abnormalities associated with reperfusion after focal ischemia of 30 to 60 minutes is not predictive of eventual tissue recovery. On the contrary, with ischemia of 30 minutes or longer, abnormal regions on DWI typically evolve into infarction and appear not to be salvageable with reperfusion alone, regardless of whether DWI abnormalities reverse during early reperfusion. This may explain why a reversal in DWI abnormalities has been convincingly shown in only a handful of stroke patients47,48 where follow-up imaging is mostly performed at time points later than 1 day. Second, after 2.5 hours of ischemia (which is still in the 3-hour time window currently used for thrombolytic therapy in humans), reperfusion in our model resulted in a rapid breakdown of the BBB associated with relatively severe vasogenic edema at ~24 to 48 hours. Potentially, the early increase in T2 signal intensity after reperfusion may thus become a useful parameter predictive of relatively severe subacute vasogenic edema. Third, our results indicate that caution should be applied when using T2WI in clinical studies to measure final infarct size. Cur-
Recently, final infarct size is typically determined on T2-weighted images, but there is still considerable debate as to which time point should be used as the end point. By choosing either 24 or 48 hours, we would have overestimated final infarct size (mainly in the 2.5-hour ischemia group); on the other hand, with 7 days as the end point, we would have underestimated final infarct size in the 30-minute ischemia group.

Finally, there are a number limitations to our study, which are of importance when trying to extrapolate from our results obtained with the rat suture model to human stroke. One important difference between the rat suture model and human stroke is that there is a sudden onset of reperfusion after removal of the intraluminal suture, whereas recanalization with either spontaneous or rtPA-induced thrombolysis in humans (and in rat models of embolic stroke4,49) probably is a comparatively slow process. In addition, with thrombolysis, partial clot fragmentation may occur and lead to the lodging of clot fragments in smaller downstream arteries and to microembolism of the peripheral vasculature.49 There may be other additional adverse pharmacological processes specific to rtPA treatment that are not encountered in mechanical occlusion-reperfusion models.50 On the other hand, the abrupt restoration of blood flow in the suture model and the resulting early postischemic hyperperfusion may exacerbate the effects of postischemic BBB disturbances, particularly with ischemia times sufficiently long to cause damage to the microvasculature.

In conclusion, this study shows that in the rat suture occlusion model, MRI characteristics of previously ischemic regions change dynamically during the reperfusion period.

The acute complete or partial reversal of DWI lesions after relatively brief ischemic periods (30 to 60 minutes) may indicate a relatively long therapeutic window, but further work is needed to establish whether secondary ischemic tissue damage can be prevented. After 2.5 hours of ischemia, BBB damage occurs early during reperfusion and invariably leads to pronounced subacute vasogenic edema, both of which can be readily assessed with MRI. Delayed ischemic damage, however, is probably of little importance when reperfusion occurs after MCAO of 2.5 hours or longer. Based on these results, the prevention of both delayed ischemic damage after brief ischemic episodes and subacute vasogenic edema after more prolonged ischemia appears to be a valuable therapeutic target, and treatment efficacy may be evaluated with serial MRI.

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References


23. van Bruggen N, van Lookeren Campagne M, Thibodeaux H, Palmer JT, Williams SP, Thomas GR. Evidence for delayed cerebral
The ability of diffusion-weighted MRI to detect early declines in the apparent diffusion coefficient (ADC) and to rapidly image acute experimental and clinical ischemic stroke represents an important advance in the diagnosis of acute stroke. However, it is now apparent from both experimental and clinical studies that acute ADC declines are potentially reversible with early intervention, with either thrombolytic or neuroprotective therapy. The reversal of initially reduced ADC values to the normal range may not invariably identify tissue salvage. Secondary declines in ADC values have been identified in both focal and global ischemia models. Pathological studies have demonstrated that these secondary ADC declines are associated with histological evidence of cellular ischemic injury and brain infarction. In stroke patients, similar secondary ADC declines after initial reversal of early ADC abnormalities by intra-arterial thrombolytic therapy were detected. The mechanisms for these secondary ADC declines in animals and humans remain uncertain, but both necrotic and apoptotic contributions have been postulated.

In this study, Neumann-Haefelin and colleagues expanded our knowledge about the phenomenon of secondary ADC declines after transient focal experimental ischemia. They observed that secondary ADC declines occurred maximally in rats subjected to 30 minutes of temporary focal ischemia and to a lesser extent in rats undergoing 60 minutes of temporary ischemia. With 2.5 hours of temporary ischemia, reduced ADC values during ischemia did not reverse with reperfusion, implying that prolonged temporary focal ischemia induces a more severe degree of initial ischemic injury that is not reversible. Interestingly, the infarct volumes at day 7 were not significantly different among the 3 groups, with a tendency toward a larger infarct volume in the 30-minute...
temporary ischemia group. Blood-brain barrier injury was not
detected in the 30-minute temporary ischemia group, whereas
it was commonly seen in the 2.5-hour group. The 60-minute
group had variable evidence of blood-brain barrier break-
down, primarily in the basal ganglia.

The phenomenon of secondary ADC declines after transient
focal brain ischemia is intriguing. Much more experimental
work is needed to characterize its pathophysiology and signifi-
cance. Secondary ADC declines, perhaps as a marker of sec-
ondary or “reperfusion” injury, may be clinically relevant in
patients who receive thrombolytic therapy. The consequences of
this secondary event on stroke outcome remain to be established,
as do the necessity and means to treat it.

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References
1. Neumann-Haefelin T, Moseley ME, Albers GW. New magnetic resonance
imaging methods for cerebrovascular disease: emerging clinical appli-
2. Dijkhuizen RM, Knollema S, van der Worp HB, Ter Horst GJ, De
Wildt DJ, Berkelbach van der Sprekel JW, Tulleken KA, Nicolay K.
Dynamics of cerebral tissue injury and perfusion after temporary hyp-
oxia-ischemia in the rat: evidence for region specific sensitivity and
3. Li F, Silva MD, Sotak CH, Fisher M. Temporal evolution of ischemic
injury evaluated with diffusion-, perfusion-, and T2-weighted MRI.
4. Li F, Liu K-F, Silva MD, Omae T, Sotak CH, Fenstermacher JD, Fisher
M. Transient and permanent resolution of ischemic lesions on
diffusion-perfusion imaging after brief periods of focal ischemia in
reversal of acute human cerebral ischemic injury shown by diffusion/
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