Diffusion Nuclear Magnetic Resonance Imaging in Experimental Stroke
Correlation With Cerebral Metabolites

Tobias Back, MD; Mathias Hoehn-Berlage, PhD;
Kanehisa Kohno, MD; Konstantin-Alexander Hossmann, PhD, MD

Background and Purpose  Diffusion-weighted nuclear magnetic resonance imaging has been shown to detect early ischemia-related alterations in experimental stroke. This raises the question of whether the observed increase in signal intensity is correlated with changes in cerebral metabolism. After middle cerebral artery occlusion, nuclear magnetic resonance diffusion images were recorded and compared with the regional concentration of cerebral metabolites and with histology of identical planes.

Methods  Seven anesthetized Fischer rats were subjected to permanent occlusion of the middle cerebral artery. T1, T2, and diffusion images (b factors ranging from 0 to 1500 s/mm2) were measured in three to five planes after 7 hours. Thereafter, brains were frozen in situ for histology and quantitative bioluminescence imaging of ATP, glucose, lactate, and for fluorescence imaging of tissue pH.

Results  Seven hours after middle cerebral artery occlusion, the apparent diffusion coefficient was reduced from 615±97×10⁻⁶ mm² s⁻¹ (contralateral brain) to 359±42×10⁻⁶ mm² s⁻¹ (ischemic brain; mean±SD, P<.01). A precise topical coincidence was demonstrated between changes in nuclear magnetic resonance diffusion images, pattern of histological damage, ATP-depleted areas, and local tissue acidosis, the lesion area amounting to between 24.1% and 27.6% of the hemisphere at the level of the caudate-putamen. The area of elevated brain lactate clearly exceeded the acidic core of the infarct and included the slightly alkaline border zone.

Conclusions  The data demonstrate that after 7-hour middle cerebral artery occlusion, the reduction of the apparent diffusion coefficient in nuclear magnetic resonance diffusion images reflects precisely the region of histological injury, breakdown of energy metabolism, and tissue acidosis. (Stroke. 1994;25:494-500.)

Key Words  clinical ischemia, focal  diagnostic imaging  nuclear magnetic resonance  rats

In experimental studies diffusion-weighted nuclear magnetic resonance (NMR) imaging (DWI) has been shown to detect cerebral ischemic lesions in the very early phase, ie, a few minutes after induction of ischemia.1,2 Regarding the diagnostic sensitivity for ischemic lesions, DWI is clearly superior to routine T1- and T2-weighted NMR imaging, which is not able to detect infarcts during the initial hours after onset of stroke.1,3 DWI has already been successfully used in studies of acute human stroke,4,5 demonstrating that the previously reported results of animal experiments are applicable to the clinical environment. Thus, for the diagnosis of stroke DWI could become the diagnostic method of choice, enabling clinicians to control the efficacy of early treatment.

Despite growing literature about the potential application of DWI, the mechanisms of the change in water proton diffusion of ischemic tissue are poorly understood. In models of middle cerebral artery (MCA) occlusion, the hyperintensity in DWI is a potentially reversible phenomenon if vessel obstruction is reversed between 30 minutes and 1 hour.6,7 This indicates that the early changes of DWI are related to reversible postischemic alterations of cerebral metabolism rather than to irreversible histological injury, which becomes manifest only after several hours of ischemia.

Several studies were able to show a close spatial correlation between altered tissue water diffusion and ischemic injury, even when only mild degrees of histological damage were observed.2,8 However, little is known about the biochemical changes accompanying or causing the changes of DWI in stroke. There is some evidence that hyperintense regions in DWI may reflect brain areas with a breakdown of energy metabolism, as suggested by measurements of reduced Na-K-ATPase activity.10,11 In a model of global cerebral ischemia, Busza et al12 reported a threshold relation between cerebral blood flow (CBF) and changes in DWI: when CBF was decreased below 15 to 20 mL·100 g⁻¹·min⁻¹, the signal intensity began to increase in parallel with ATP depletion.12 This raises the question of whether the relation between energy metabolism and tissue water diffusion also applies for the more heterogenous pattern of focal ischemia. Other pathogenic factors such as glucose deprivation, tissue acidosis, or lactate accumulation may contribute to ischemia-related alterations in DWI as well.

For this reason, we evaluated the spatial correlation between the regional distribution of cerebral metabolites and the postischemic changes in NMR diffusion images in a model of experimental stroke. Quantitative images of the relaxation times T1 and T2 and of the apparent diffusion coefficient (ADC) were measured.
after 7 hours following MCA occlusion. The NMR measurements at the end of the observation period were correlated with bioluminescence and fluorescence images of ATP, glucose, lactate, and tissue pH obtained from brain sections in identical planes.

### Materials and Methods

#### Experimental Protocol
Seven male Fischer rats of the CDF 344 strain (280 to 370 g) underwent MCA occlusion for 7 hours. Anesthesia was induced via a face mask with 1.5% halothane and 70% N2O (remainder oxygen). A femoral vein and artery were cannulated for continuous measurement of arterial blood pressure, intravenous administration of drugs and fluid, and to obtain samples for blood gas analysis. A rectal temperature probe was inserted and connected to a feedback-controlled heating pad to maintain normal body temperature.

The animals were mounted in a stereotaxic frame, and the stem of the left MCA was exposed by subtemporal craniotomy and electrocoagulated with a microforceps proximal or distal to the crossing of the olfactory tract. Depending on the site of coagulation, basal ganglia infarcts (n=3) or larger hemispheric infarcts also involving the frontotemporal cortex (n=4) were induced. A tracheal tube was inserted, and the animals were immunoanized with pancuronium bromide (0.3 mg/kg) and artificially ventilated. Halothane concentration was reduced to 0.8%; nitrous oxide concentration remained at 70%.

For the NMR experiment, the animals were fixed in a nonmagnetic head holder. Arterial blood pressure was continuously recorded, and arterial blood samples were withdrawn every 30 minutes to measure blood gases, plasma glucose, and hematocrit. The rectal temperature probe was connected to a feedback-controlled water jacket covering the body of the animal. Body temperature was kept constant at 36.4±1.3°C throughout the experimental period.

#### Nuclear Magnetic Resonance Measurements
A BIOSPEC 4.7-T system (Bruker, Karlsruhe, FRG) was used. The system was equipped with an Alderman-Grant resonator for excitation and signal reception and a 15-cm-diameter mini-imaging gradient set (maximum gradient strength, 120 mT/m). Four animals were investigated after the system had been retrofitted with an actively shielded gradient set (21-cm inner diameter; 100 mT/m; rise time, =250 μseconds; Bruker) replacing the mini-imaging gradient set. MULTISlice sagittal pilot scans (repetition time [TR], 600 milliseconds; echo time [TE], 18 milliseconds) were recorded for selection of the optimal location of the coronal sections. The central slice was positioned approximately 5 mm posterior to the rhinal fissure. For all experiments, slice thickness was 1 mm, the gap between slices was 0.75 mm, and the field of view was 6 cm. The image matrix was always 128×256.

T1 and T2 were recorded in two coronal slices through the center of the infarct. The simultaneous measurement of T1 and T2 was performed using a modified multiecho 2DFT Carr-Purcell-Meiboom-Gill sequence, as described in detail elsewhere.15,16 The sequence consisted of a train of 20 to 32 echoes (TR1, 3200 milliseconds; TE, 18 or 13 milliseconds), followed by a second excitation to obtain a second train of eight echoes with identical TE but a short TR time (TR2, 600 milliseconds). Two phase-cycle steps resulted in 36 minutes of experimental time.

The ADC was measured in the same position, using a multislice Stejskal-Tanner–type pulsed-gradient, spin-echo method (TR, 3000 milliseconds). TE was between 32 and 55 milliseconds to minimize a T1-dependent loss in signal to noise. At a TE of 32 milliseconds, the gradient power supply could produce b factors of up to 1500 s/mm2. The slight variation in TE has no significant effect on the contrast situation. Because T1 in the infarct area increases with time, an increase of TE will lead to an increasing contrast effect between infarct and normal brain, in parallel to the contrast development produced by the diffusion difference. Therefore, no adverse effect needs to be considered, but any T2 effect will only further increase the contrast in the DWI.

For quantitative determination of ADC, four to six sets of images were recorded with the b factor ranging equidistantly between 0 and 1500 s/mm2. The diffusion-encoding gradient was aligned parallel to the read direction of the imaging gradients, ie, left-to-right or up-down within the image plane. The variation in direction of diffusion-encoding gradient had no visible effect on the ischemia-induced changes, indicating that diffusion anisotropy in the tissues under investigation, cortex and caudate-putamen, could be neglected within the accuracy of the present data. Therefore, no separation of the calculated ADC depending on direction of diffusion-encoding gradient was tried. Images were always recorded sequentially with increasing b factors.

#### Computation of Quantitative Nuclear Magnetic Resonance Parameter Images
From the magnitude-calculated images the mean value m and the standard deviation s of the background noise were determined in each individual image. T1 determination was modified from the echoes of the first multiecho train with signal intensity above the noise level (NL) as NL=m+3s, using the software system RAMSES.17,18 The data were analyzed for biexponential behavior with a modified Marquardt algorithm.19 For the determination of T2, the theoretical signal intensity ratio Q™ was computed as follows

\[
Q™=\left(1-\exp\left(-\frac{TR}{T2}\right)\right) / \left(1-\exp\left(-\frac{TR}{T1}\right)\right)
\]

for a T1 range between 30 and 3000 milliseconds and stored in a look-up table. The ratios Q™, where

\[
Q=\text{signal}(1\text{st echo train with } TR) / \text{signal}(2\text{nd echo train with } TR)
\]

with 1≤s≤8 were calculated for each pixel (provided that every signal, from both echo trains was above the noise level). These experimentally measured Q™ values were compared with the theoretical values Q™ obtained in the look-up table to yield the corresponding experimental T1. The mean of the individual T1™ values as obtained from the eight ratios Q™ was calculated as the final T1™ value for all pixels.15,16

Calculation of a quantitative parameter image required 20 minutes for T1™ but only 12 seconds for T2™ on a VAX 3200 workstation (Digital Equipment Corporation). ADC was calculated within 5 seconds from the four to six images with increasing diffusion weighting. For this purpose, the algorithm used for the T2™ determination was modified in the RAMSES system to allow for each pixel a single-exponential fit of the function:

\[
S(b)=S(b=0) \cdot \exp(-b \cdot ADC)
\]

where S is the signal intensity and b the gradient strength.20 The quantitative parameter images were transferred onto a Macintosh Quadra 900 and evaluated using the public domain image processing program IMAGE. Based on the coronal ADC images, regions of interest (4×4 pixels) from the ischemic frontotemporal cortex and caudate-putamen region and equivalent positions on the contralateral side were chosen for evaluation of the three quantitative parameter values.

#### Histology and Regional Biochemistry
Seven hours after induction of MCA occlusion, brains were frozen in situ with liquid nitrogen. Brains were cut at ~20°C into 20-μm coronal sections in the same planes as the NMR images. Cryostat sections were processed by the umbelliferone fluorescence method to evaluate the regional distribution of tissue pH and by substrate-specific bioluminescence for the...
TABLE 1. Mean Physiological Variables During 7 Hours of Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP, mm Hg</td>
<td>133±33</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>36.4±1.3</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.45±0.12</td>
</tr>
<tr>
<td>Arterial PaO₂, mm Hg</td>
<td>37±15</td>
</tr>
<tr>
<td>Arterial PaO₂, mm Hg</td>
<td>145±46</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>186±29</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>37±7</td>
</tr>
</tbody>
</table>

MABP indicates mean arterial blood pressure. Values are mean±SD from 39 measurements in seven animals.

Regional distributions of ATP, lactate, and glucose.22-24 Adjacent sections were stained with hematoxylin-eosin to evaluate ischemic tissue necrosis. The bioluminescence images were calibrated by measuring metabolite concentrations in tissue samples taken from the remaining parts of the brain.

Data Analysis and Statistics

Data are expressed as mean±SD. Differences between experimental parameters were tested for significance by ANOVA. For multiple comparisons Fisher's protected least-squares difference test was used. Statistical differences between ischemic and contralateral normal brain were evaluated by the paired t test. A level of P<.05 was accepted as significant unless stated otherwise. The lesion size was evaluated at the level of the caudate-putamen and expressed as percentage of hemisphere. The following lesion criteria were used: paleness in histological sections, increase of signal intensity in DWI relative to the contralateral side by more than 10% (at the highest b factor), ATP reduction below 1.0 mmol/kg, pH decrease below 6.4, glucose reduction below 0.8 mmol/kg, and lactate increase to above 6 mmol/kg. Image analysis and processing were carried out on a Macintosh Illfx computer, using the program IMAGE.

Results

Physiological Variables

Table 1 summarizes the physiological parameters measured during the 7 hours of MCA occlusion. During the whole length of the observation period, all variables were kept in the normal range and did not change significantly.

Nuclear Magnetic Resonance Measurements

The data are summarized in Fig 1 and Table 2. At 7 hours after occlusion, ADC in the core of infarction was reduced from 615±97×10⁻⁶ mm²·s⁻¹ (contralateral brain) to 359±42×10⁻⁶ mm²·s⁻¹ (infarcted basal ganglia, P<.01). In parallel, T₁ increased from 886±91 milliseconds to 1014±116 milliseconds, and T₂ from 73±2 milliseconds to 90±7 milliseconds (P<.01) (Fig 1, Table 2). In infarcted cortical regions, ADC was reduced from 676±69×10⁻⁶ mm²·s⁻¹ to 528±94×10⁻⁶ mm²·s⁻¹ (P<.01); T₁ and T₂ did not change significantly. A typical example is shown in Fig 1. After 7 hours of ischemia, the infarct was clearly visible in ADC and T₂ maps, while the T₁ image showed less pronounced changes. In DWI, 27.6±17.3% of the affected hemisphere displayed an increase in signal intensity at the level of the caudate-putamen (Fig 2). The high scatter derived from the variability in infarct size (see below).

Histology

On histological sections corresponding to the planes of NMR measurements, infarcts were well demarcated after 7-hour MCA occlusion (Fig 3). In three animals,
infarcts were restricted to the central parts of caudate-putamen, but in four animals infarcts also involved lateral basal ganglia and frontotemporal cortex. The mean area of tissue necrosis amounted to 24.9±18.7% of hemisphere and corresponded precisely to that of increased signal intensity in DWI (Figs 2 and 3).

**Distribution of Cerebral Metabolites**

In ATP-specific bioluminescence images, the area of ATP depletion corresponded to that of histological injury, hyperintensity in DWI, and reduced ADC, respectively. The size of ATP depletion amounted to 25.6±19.9% (Fig 2). Mean ATP concentration in the infarct was reduced from 3.1±0.7 mmol/kg (contralateral side) to 0.4±0.5 mmol/kg (P<.001) in the infarct (Table 2). In six of seven animals, the peri-infarct border zone exhibited a normal concentration of high-energy phosphates.

Fluorescence pH images revealed well-delineated areas of tissue acidosis coinciding topically with those of breakdown of energy metabolism and of changes in histology and DWI. The lesion size in pH images was 24.1±17.1% of hemisphere. Mean pH decreased from 6.90±0.14 to 6.09±0.19 (P<.001) in the infarct (Table 2). In the peri-infarct border zone, an alkaline pH shift (pH 7.2 to 7.5) was detected (Fig 3).

Glucose concentration was variable within the infarcted tissue. In large hemispheric infarcts involving frontotemporal cortex (n=4), glucose was reduced to approximately 0.5 mmol/kg in the center of infarction (basal ganglia). In the peripheral zone glucose content was normal. Animals with isolated infarcts of the caudate-putamen (n=3) did not show substantial change in glucose concentration. The mean area of depleted glucose was 4.2±7.3% of hemisphere (P<.05, significantly different from size of histological injury) (Fig 2).

Tissue lactate increased to approximately 9 mmol/kg in an area that clearly exceeded infarct size by spreading into the alkaline peri-infarct border zone. The border of elevated lactate showed a diffuse transition with no sharp delineation from the surrounding normal tissue. In the lactate images, 37.3±17.9% of the affected hemisphere had values above 6 mmol/kg (P<.05, significantly different from size of histological injury) (Figs 2 and 3).

**Discussion**

The present method of subtemporal MCA coagulation produces two types of infarcts: a small type located...
edema is usually associated with an increase of ADC,26 because of constrained collateral blood supply, these infarct types mimic autochthonous thrombotic infarcts of the lenticulostriate artery or embolic MCA territory infarcts in human stroke, respectively. The regional changes in ADC maps coincided precisely with the histological pattern of both types of ischemic injury, which is in good agreement with the previously documented spatial correlation between signal hyperintensity in DWI and histology as obtained between 2 hours and 7 days after onset of the insult.5-8,22

The most pronounced reduction in ADC by 41±6% was in the infarcted caudate-putamen, which is also in line with previous reports.2,5 In the affected cortical areas of the large hemispheric MCA territory infarcts, ADC declined only by 23±7%, but the decrease was significant, in contrast to T₁ and T₂ images that did not significantly change. This confirms that quantitative maps of water diffusion are not only superior in detecting early ischemia-related changes but also have a higher sensitivity for ischemic cortical lesions at later stages of the ischemic insult.22,25

To the best of our knowledge, this is the first demonstration of a precise topical correlation between the changes in DWI, histological damage, breakdown of energy metabolism, and local tissue acidosis during the subacute phase of experimental stroke. In regard to ATP, pH, and DWI, correlation was equally good in the small centrally located and in the large hemispheric infarcts, indicating that after 7 hours infarcts are already sharply demarcated from the normal tissue.

The correlation between the depletion of high-energy phosphates and acidosis is due to the combined effect of energy failure (proton production via ATP hydrolysis), reduced CO₂ clearance (severe blood flow reduction), and decreased cellular extrusion of protons (failure of Na⁺/H⁺ and Cl⁻/HCO₃⁻ antiporter systems). In addition, the increase of lactic acid to approximately 9.0 mmol/kg contributes to the accumulation of protons and explains that tissue pH was reduced by almost 1 unit. In contrast to ATP and pH, the pattern of glucose content differed between the two infarct types. In the small infract glucose concentration was normal, whereas in the large hemispheric infract glucose was decreased in the central parts, indicating that collateral blood flow was unable to supply glucose into the ischemic core. In the peri-infarct border zone the metabolic pattern was again similar in all animals: ATP and glucose concentrations were close to normal, but the tissue lactate was almost as high as in the infract core, and tissue pH showed an alkaline shift by 0.2 to 0.5 units.

A possible explanation for the dissociation between lactate and pH is a spread of (alkaline) lactate salt from the infract core into the surrounding tissue in analogy to peritumoral edema, which also exhibits increased lactate levels in the absence of acidosis. However, because of the expansion of extracellular space, this type of edema is usually associated with an increase of ADC,26 not observed in the present study. An alternative interpretation is reactive alkalosis following a transient episode of metabolic acidosis. In the periphery of infarcts, spreading depression-like depolarizations occur27,28 associated with a marked stimulation of metabolism.29-31 Because of constrained collateral blood supply, these episodes result in relative hypoxia,32 which may stimulate anaerobic glycolysis33 and lactate accumulation.33-35

Obviously, these episodes are associated with a decline of ATP and acidosis,33,35 but it has been suggested that regeneration of ATP (which consumes protons) and an overcompensation of the Na⁺/H⁺ antiporter may cause reactive alkalinization.36 This interpretation would also be in line with the previously observed tissue alkalinization following reversible focal or global ischemia.30-39 Recent repetitive spectroscopic imaging studies on the thread occlusion model for MCA occlusion40 have given new insight into the temporal evolution of lactate production and its regional distribution across the affected hemisphere. Within minutes after occlusion, a rise in lactate was observed in that study. Detailed information concerning the evolution of lactate after occlusion is expected from the continuation of such spectroscopic imaging investigations.

In contrast to the distribution of lactate, the changes in DWI were closely related to the area of ATP depletion. This confirms earlier suggestions that the alteration in the diffusion properties of water are directly or indirectly related to energy-dependent membrane functions.10,12,14 One of the various mechanisms that have been discussed is the narrowing of the intracellular space. In fact, extracellular space begins to shrink within a few minutes after vascular occlusion42-44 and declines to almost 50% of control within 30 minutes. This observation is in line with the interpretation by Benveniste et al.45 and van Zijl et al.46 They suggest that the macroscopically observed ADC is the volume-weighted average of the two microscopic diffusion constants of the intracellular (Dᵈᵢᵣ) and the extracellular (Dᵈₑᵣ) compartments. In the ischemic region water shifts into the cells, thereby also shifting the volume-weighted average of the diffusion constants toward the intracellular constant, which is believed to be smaller than the extracellular constant by approximately one order of magnitude.45,46

However, threshold determinations revealed that extracellular shrinkage occurs at substantially higher flow values than that required for maintaining energy metabolism.44,47,48 This would imply that the region of altered DWI should be larger than that of deranged energy depletion. Obviously, this was not the case in the present situation, but recently we observed that there is, in fact, such a dissociation at earlier stages of infarct development (K.K., unpublished data, 1993). Thus, it might be argued that the severe reduction of the microcirculatory flow will, to some extent, contribute to the observed decrease in ADC on MCA occlusion.

The alternative explanation for the reduction of ADC would be a restriction of the intracellular water diffusion. Because the intracellular volume increases during ischemia, this restriction could only be caused by a decline of the water permeability of the plasma membrane.49 Further support for this interpretation comes from recent data on ADC studies of healthy and infarcted brain of the gerbil.50 In this study non-monoeponential decay of the NMR signal amplitude was observed when the diffusion-encoding gradient strength was kept constant while the diffusion time was varied. Such a decline could be brought about by the reduced ion traffic across the membrane after cessation of the function of ion exchange pumps because this possibly also results in a reduced exchange of osmotically obliged...
Acknowledgments

This study was supported by the Deutsche Forschungsgemeinschaft (SFB 194/B1) and by the Ministerium für Wissenschaft und Forschung of Nordrhein-Westfalen. We are grateful for the support by Dr M. Eis, Mrs U. Uhlenuken, and Mr P. Spiegelsberg. Also, we thank Mrs A. Koll for technical assistance and Mrs U. Beckmann for excellent help with the bioluminescence and fluorescence techniques.

References


Diffusion-weighted proton magnetic resonance imaging (DWI) has shown great potential in the study of stroke. This recently developed technology can detect the onset of ischemia within minutes, providing exquisite detail in high-resolution images of evolving physiological processes. The sensitivity of the technique is based on the movement of water molecules (i.e., the apparent diffusion coefficient of water [ADC]); however, the underlying biologic mechanism(s) responsible for the changes in ADC that occur in ischemic tissue are currently unknown and remain an active area of investigation. Previous studies have shown that ADC declines shortly after the onset of cerebral ischemia, then gradually returns to normal or elevated values over time. The acute decline in ADC has been related to metabolic breakdown, loss of Na-K homeostasis, and a threshold level of cerebral blood flow, all of which are associated with ischemic damage. Regional DWI changes in experimental stroke have been confirmed histologically.

The study of Back et al supplies further evidence toward the validity of DWI as an intrinsic marker of acute stroke by providing the first precise regional correlation of DWI changes with established histopathological measures of ischemic cellular damage. In particular, a one-on-one topical correlation between altered levels of ATP, pH, and ADC is demonstrated in rats 7 hours after occlusion of the middle cerebral artery. Studies of this nature are fundamental to our understanding of the movement of water in stroke-affected tissue and further illustrate the importance of DWI as it relates to the pathophysiology of ischemic brain damage.

Although some technical difficulties exist, the implementation of DWI in the clinical realm is currently under way, and preliminary studies in human stroke have demonstrated findings similar to those obtained in animals. Future expectations for DWI include the possibility of differentiating between permanent and reversible ischemic damage, as well as assessment and guidance of therapeutic intervention. The challenge at hand is to extend the application of this technology to the realm of human brain imaging.

Robert A. Knight, PhD, Guest Editor Center for Stroke Research Department of Neurology Henry Ford Hospital Detroit, Mich
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T Back, M Hoehn-Berlage, K Kohno and K A Hossmann

Stroke. 1994;25:494-500
doi: 10.1161/01.STR.25.2.494

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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