Temporal Evolution of Ischemic Damage in Rat Brain Measured by Proton Nuclear Magnetic Resonance Imaging

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We studied the effect of focal cerebral ischemia on the “state” of brain water using proton nuclear magnetic resonance imaging. Focal cerebral ischemia was induced in five halothane-anesthetized rats via tandem occlusion of the left common carotid artery and the left middle cerebral artery. The proton transverse relaxation time, the proton density, and the water diffusion coefficient were measured at various times from the same region of brain tissue from 1.5 to 168 hours after occlusion. Early measurements indicated significant changes in the transverse relaxation time (p=0.004) and water diffusion coefficient (p<=0.002) of ischemic brain tissue compared with a homologous region from the contralateral hemisphere. However, the transverse relaxation time, proton density, and water diffusion coefficient in ischemic brain tissue showed different temporal evolutions over the study period. Diffusion coefficient weighting was superior to relaxation time and proton density weighting for the visualization of early cerebral ischemia. Our data suggest that nuclear magnetic resonance imaging is sensitive in detecting changes in proton-associated parameters during early cerebral ischemia and confirm significant changes (p<0.01) in the temporal evolution of transverse relaxation times, proton densities, and diffusion coefficients following middle cerebral artery occlusion. (Stroke 1991;22:802–808)

Nuclear magnetic resonance (NMR) offers the ability to assess noninvasively several physical parameters that are directly related to the amount and “state” of water in brain tissue. The spin–spin relaxation time (T2) is exquisitely sensitive to the relatively slow motions (i.e., rotation of proteins) of the system. Proton density (ρ) images reveal the spatial distribution of the number of NMR-visible nuclei in the tissue. More recently, NMR has been used to generate images the contrast of which is based on the diffusion coefficient of water (Dw).1–4

We document the temporal evolution of changes in T2, ρ, and Dw following occlusion of the middle cerebral artery (MCA) in rats. These measurements provide new information regarding the relative time course of changes in these parameters in experimental ischemia based on the physical properties of water. Characterization of the time course of these NMR-measurable parameters, for both normal and ischemic brain tissue, may provide new insight into the mechanisms responsible for ischemic brain damage and cerebral edema. Comparison of the various types of images may be clinically useful when the physical basis of these changes is more thoroughly understood.

Materials and Methods

Five fasted adult male Fisher rats weighing 175–250 g were anesthetized with an inhaled mixture of 69% N2O, 30% O2, and 1.0–1.5% halothane. The rats were allowed to breathe spontaneously throughout all surgical and NMR procedures. Focal cerebral ischemia was induced using tandem occlusion of the left common carotid artery and the left MCA, similar to that described by Brint et al.5 Briefly, the left common carotid artery was isolated, tied, and cut between two sutures. An incision was then made in the left temporoparietal region, and the temporalis muscle and lower jaw bone were partially removed. The MCA was isolated through a small (1–2 mm diameter) burr hole drilled approximately 1 mm rostral to the fusion of the zygomatic arch with the squamosal bone. Under direct visualization with a
dissecting microscope (Carl Zeiss, Inc., Thornwood, N.Y.) the dura was retracted, thereby exposing the left MCA. The MCA was then coagulated using bipolar forceps (Codman & Shurtleff, Inc., Randolph, Mass.) and cut at the level of the rhinal fissure. Absorbable gelatin sponge (The Upjohn Co., Kalamazoo, Mich.) was then placed over the area of the craniotomy, and the incision was sutured shut.

Immediately following surgery the rat was placed in a Plexiglas holder. This holder consists of a 20-cm-diameter radiofrequency (rf) shield and a Plexiglas frame with a water-heated support platform for the animal's body. The holder is equipped with an rf transmit/receive coil tuned to the $^1$H resonant frequency (80.3 MHz) and stereotactic ear bars to minimize movement during the imaging procedure.

The rf coil was constructed from two 5-cm-diameter double-turn surface coils wired in parallel and rigidly fixed in a "pseudo" Helmholtz configuration. The rat's head was placed inside this coil and held in place using the ear bars and an adjustable mouthpiece. After positioning the animal inside the holder, the entire setup was placed inside the magnet. The concentration of halothane was decreased to 0.75%, and rectal temperature was maintained using a feedback-controlled water bath at 36–37°C.

All images were acquired using a 1.89-T, 60-cm-bore superconducting magnet (Oxford Magnet Technologies, Eynsham, England) interfaced to a Biospec I spectrometer (Bruker Instruments, Billerica, Mass.). A 20-cm-bore gradient coil insert capable of producing magnetic field gradients up to 2 G/cm was used to achieve the spatial resolution necessary to resolve structures within the rat brain. The NMR regimen used produced images with a 6 cm field of view. The images were reconstructed using a $128 \times 128$ matrix, which translates to an in-plane resolution of approximately 0.47 mm/pixel. The slice thickness was 3 mm.

Accurate, reproducible positioning of the rat on a daily basis was essential to ensure that the image slices were always taken at the same level. A modified fast low angle shot* (FLASH) imaging sequence was used to align the animal correctly in the imaging system. This sequence produces two simultaneously acquired images from perpendicular planes (Figure 1). Saturation effects at the intersection of the two selected planes produce the dark band that serves as a marker indicating the axial position, angle, and thickness of the image slice through the brain. The entire sequence requires approximately 1 minute, thus allowing rapid repositioning of the rat ($\pm 0.5$ mm) in an iterative manner. The animal's position was adjusted until the image slice was 5 mm posterior to the rhinal fissure, with the head of the rat held in a flat skull position. The prolonged surgical procedure combined with the necessity for accurate positioning within the magnet precluded NMR measurements $\leq$1.5 hours after MCA occlusion.

We measured $T_2$ using standard two-dimensional Fourier transform (2DFT) spin/echo NMR imaging with an incremented echo time (TE) and a fixed repetition time (TR). A series of five images was obtained using TEs of 10, 29, 59, 109, and 209 msec and a TR of 3 seconds. Each image in the series required approximately 6.5 minutes for completion, resulting in a total experimental time of approximately 33 minutes. Because the spin-lattice relaxation time ($T_1$) of normal and ischemic rat brain varies from 0.7 to 1.5 seconds, a TR of 3 seconds results in slight distortion of the slice profile. Partial saturation of the NMR signal, however, does not affect the measurement since $T_2$ is calculated using a series of experiments that all have identical $T_1$ weighting. The images were analyzed on a pixel-by-pixel basis using the least-squares fit to a straight line on a plot of the natural logarithm of the normalized image intensity versus TE. Pixels were then combined to produce an average $T_2$ value from a region of interest (ROI) within an isointense area of brain tissue on the NMR images.
FIGURE 2. T2-weighted nuclear magnetic resonance images (echo time = 59 msec, repetition time = 3 seconds) of coronal sections through rat brain 2, 4, 8, 24, 48, and 168 hours after middle cerebral artery occlusion (MCAO). Ischemic region (right side of images) is delineated by evolution of hyperintensity during study period.

In addition to the T2 information obtained from this series of images, the first image in the sequence (TE = 10 msec, TR = 3 sec) was used as a relative measure of p. Image intensity data were obtained by selecting the same ROI at each time the rat was studied. A homologous ROI on the contralateral side of the brain was also measured, and the data are reported as p in the ischemic brain tissue divided by p in the contralateral brain tissue (signal intensity ratio).

The value of Dw was measured using the method described by LeBihan et al. Standard 2DFT spin/echo NMR imaging was modified to include two 60-msec diffusion-weighting gradient pulses on either side of the refocusing 180° rf pulse. A TE of 130 msec was used with a TR of 1.5 seconds. The diffusion-weighting gradient was increased in a nonlinear manner from 0 to approximately 1.3 G/cm to obtain a series of seven images. Taking into account the timing of the imaging gradients, the equivalent gradient b values for this sequence1,9 were 0, 18, 73, 292, 659, 1179, and 1825 sec/mm². Each image was signal-averaged twice to improve the signal-to-noise ratio, and thus each image required a total scan time of 7 minutes for completion. The total experimental time for the entire series was therefore approximately 50 minutes. The TR and TE values used to produce this series of diffusion-weighted images results in some additional T1 and T2 weighting of the image intensity. Since TR and TE were held constant as the diffusion-weighting gradient was varied, all images in the series contain equal T1 and T2 contributions. The calculation of Dw requires that each image in the series be normalized to a baseline image obtained with b = 0 sec/mm²; therefore, the additional T1 and T2 weighting factors cancel and do not affect the value of Dw. Images of phantoms of water and acetone were used to verify the accuracy of the Dw measurement by comparison with published values. Images were analyzed on a pixel-by-pixel basis using the least-squares fit to a straight line on a plot of the natural logarithm of the normalized image intensity versus the gradient b value. Pixels were combined to produce an average Dw value using an ROI analogous to that used in the T2 measurement. Images obtained using gradient b values of 18 and 73 sec/mm² were not used in the calculation of Dw to avoid possible contamination from microcirculatory effects.1,10

The ROIs were specified for each rat from T2-weighted images obtained at 24 hours. At this time the lesion could be clearly identified as a zone of hyperintensity on the right side of the images (corresponding to the left side of the animal). The size of the ROI was adjusted to obtain information from as many pixels as possible without extending beyond the hyperintense zone. A homologous ROI was defined.
for the contralateral hemisphere. Using the sagittal sinus and the midline of the brain as reference points, we obtained measurements from identical ROIs for each rat at each time.

The evolution of NMR parameter changes were followed both acutely and chronically from 1.5 to 168 hours following MCA occlusion. T2- and Dw-weighted image sets were obtained at various times after animal preparation and positioning (1.5-2.0 hours after MCA occlusion) until approximately 8 hours after MCA occlusion. Following this procedure, the rat was allowed to recover and was given free access to food and water for the duration of the experiment. The rats were reanesthetized for subsequent measurements 24, 48, 96, and 168 hours after MCA occlusion.

The results were analyzed for statistical significance using paired t tests. T2 and Dw values for the ischemic and contralateral (control) ROIs were compared at each time. T2, Dw, and the signal intensity ratio were also analyzed by comparing the value obtained at each time with the initial measurement. Due to the multiple testing involved in this analysis, a conservative probability value (p<0.01) was used to indicate significance.

Results

Figure 2 shows a sequence of T2-weighted images from a representative rat 2, 4, 8, 24, 48, and 168 hours following MCA occlusion. The images clearly depict the evolution of a hyperintense region that is maximum in size and intensity at 24 hours. Average T2 values are shown as a function of time in Figure 3. Significant differences for the ischemic and control ROIs were found at all times except 168 hours, when only a marginal level of significance was detected (p=0.017). The control T2 value remained constant for up to 168 hours after MCA occlusion. However, T2 in ischemic brain increased steadily over the first 8 hours following MCA occlusion and reached a maximum of approximately 160% of control values at 18-24 hours. Comparison of initial T2 values from the ischemic ROI with later measurements showed significant differences at 18-24 and 48-72 hours. The T2 value in ischemic brain then gradually declined between 48 and 168 hours but did not return to the control value.

Figure 4 shows Dw-weighted images obtained from the same rat as in Figure 2. The ischemic region is apparent as a zone of hyperintensity on the right side of the images. The hyperintense region expanded during the first 24 hours; image contrast subsequently decreased. Average Dw values are presented as a
function of time in Figure 5. Significant differences for the ischemic and control ROIs were detected at 1.5–4, 4–8, and 18–24 hours, indicating a marked reduction in $D_w$ to approximately 50% of the control values. $D_w$ values in ischemic brain increased toward control values between 24 and 48 hours, and there were no significant differences after 48 hours. No significant differences in $D_w$ for the control ROI were found over the course of the experiment.

The $p$-weighted images (Figure 6) were obtained with a TR of 3 seconds and a TE of 10 msec and are, therefore, slightly $T_1$- and $T_2$-weighted. Increased $p$ in ischemic brain was detected by 24 hours, and values remained elevated for up to 96 hours after MCA occlusion before returning to control values at 168 hours. Figure 7 is a plot of the average signal intensity ratio versus time. The graph shows an increase in the NMR-visible water density of up to 13% in the ischemic ROI; the increase was maximal several days after MCA occlusion and then the ratio declined toward control values. Signal intensity ratios were significantly elevated compared with the initial measurement at 48–72 and 96–120 hours, with marginal significance ($p=0.014$) at 18–24 hours.

Discussion
Recent studies have demonstrated that $T_2$ values are markedly increased early during the course of ischemia and are the basis for NMR imaging being a more sensitive indicator of acute stroke than computed tomography. This increase in $T_2$ has been associated with the development of edema, but an explicit relation has not been demonstrated. Tissue water is believed to exist in multiple states, with much of the water existing in a structured or “bound” form and the remainder existing as bulk or “free” water. The free water fraction has the longest $T_2$ value; therefore, the $T_2$ value calculated for a tissue reflects the mean $T_2$ values of different water components within each fraction. The rationale for a relation between $T_2$ and edema is based on the fact that pure water has a relatively long $T_2$ compared with tissue water. Since the changes that occur in cerebral ischemia and its subsequent edema involve many factors besides increased water content, the analogy between edema and pure water is used only to illustrate that an increased tissue water content alone can cause a certain degree of $T_2$ lengthening. An increase in the tissue water content should, therefore, increase the observed $T_2$ value.

Other investigators, however, have indicated that increased tissue water content alone cannot be en-
tirely responsible for the observed increase in $T_2$ associated with cerebral edema and suggest that changes in water structure also play a role.\textsuperscript{1,21,22} Unger et al\textsuperscript{22} have demonstrated that boiling an egg for different times can produce large variations in the $T_2$ value of egg white although the actual water content changes little. Other experiments, both in vitro\textsuperscript{23} and in vivo,\textsuperscript{21} supporting this hypothesis have shown that changes in water content alone are insufficient to explain the observed variations in $T_2$.

In our study, the $T_2$ value of acutely ischemic brain tissue was significantly elevated at the earliest time studied and increased further, reaching a maximum at 24 hours before declining toward a persistently elevated value at 168 hours after MCA occlusion. If this observed increase in $T_2$ is a result of cerebral edema, it might be expected that $\rho$-weighted images would also demonstrate an increased signal intensity in the region of increased $T_2$ values. Such an increase was not apparent until several days after MCA occlusion. The increased $T_2$ values seen at times less than 4 hours might be attributed to changes in $\rho$ within the variance of the measurement; however, this fails to explain the similar $T_2$ values observed at 4–8 and 96–120 hours, when $\rho$ values were significantly different, or the differences in $T_2$ values at 18–24 and 96–120 hours, when $\rho$ values were similar. The acute changes in $\rho$ following the onset of ischemia were, therefore, insufficient to explain the magnitude of the increase in $T_2$ values, leading us to conclude that although an increased $\rho$ observed more chronically may contribute to long-term elevations of $T_2$ values, the temporal evolutions of $T_2$ and $\rho$ do not demonstrate the necessary conditions for a simple relation between $T_2$, $\rho$, and edema.

There is considerable evidence that the exchange of spins between free and bound water has a dramatic effect on NMR relaxation processes in vivo.\textsuperscript{24,25} A change in the relative fractions of these water compartments without a net change in the total amount of water during ischemia may contribute to the observed changes in $T_2$. Accordingly, the prolongation of $T_2$ in ischemic brain tissue observed may be a direct result of a decrease in the amount of bound water. This is consistent with the commonly held belief that bound water fractions are reduced in disease states.\textsuperscript{25}

Integral to the investigation of the role of water in stroke is the assessment of $D_w$. $D_w$ reflects mainly the degree of translational motion of intracellular water since this forms the bulk of the NMR-visible proton signal in brain tissue. Moseley et al\textsuperscript{2} recently reported $D_w$ values of 3.1–3.4 x 10$^{-4}$ mm$^2$/sec for ischemic brain and 9.1 x 10$^{-4}$ mm$^2$/sec for normal (corresponding contralateral hemisphere) brain within 8 hours following MCA occlusion in cats. Their results demonstrated an elevated average signal intensity ratio (SIR, $D_w$ in ischemic brain/$D_w$ in normal brain) of 52% within 1 hour after MCA occlusion; SIR increased to approximately 75% by 8 hours. $T_2$-weighted images from their study, however, failed to demonstrate a significant increase in SIR until 6–8 hours after occlusion.

Our results also demonstrate a significant reduction in $D_w$ ($p=0.002$) for ischemic brain tissue at the earliest time after MCA occlusion. This reduction was followed by an increase toward control values between 24 and 48 hours after occlusion. The cause of this reduced $D_w$ during the acute stages of ischemia is unknown. Possible explanations include the restriction of water to limited volumes that are smaller than the average translational displacement that normally occurs or an increase in the frequency of interactions with macromolecules or disrupted cell structures that would act to retard molecular motion.

Reduction in $D_w$ could also be caused by an increase in magnetic susceptibility-induced field gradients in ischemic tissue associated with reduced tissue oxygenation.\textsuperscript{26} Finally, the change in $D_w$ early during ischemia may result from the effects of acute cytotoxic edema. Redistribution of extracellular to intracellular water would reduce $D_w$ if intracellular water has a lower diffusion constant than extracellular water. However, no evidence has yet been published to support this hypothesis.

The temporal evolution of changes in $T_2$ and $D_w$ shows that these parameters begin to return toward normal values after 24 hours following MCA occlusion. Proton density measurements also demonstrate a similar trend after 48 hours. One possible explanation for the return of these parameters toward normal values may be the infiltration or activation of inflammatory cells into the ischemically damaged region.\textsuperscript{27,28} Further histological evaluation is needed to substantiate this hypothesis.

Our early results confirm significant changes in the temporal evolution of $T_2$, $D_w$ and $\rho$ following MCA occlusion. Future histopathological correlation of equivalent brain sections from sacrificed animals may provide direct evidence to support one or more of the proposed hypotheses for changes in $D_w$. Once these changes are understood, it may be possible to predict the severity of ischemic brain damage in human stroke from early magnetic resonance imaging. Concomitant benefits to patient treatment and the evaluation of drug therapies might then be realized.

Acknowledgment

The authors thank Dr. J.P. Windham for assisting with the image and error analysis algorithms.

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KEY WORDS • brain edema • cerebral ischemia • magnetic resonance imaging • rats
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*Stroke*. 1991;22:802-808
doi: 10.1161/01.STR.22.6.802

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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