Magnetic Resonance Imaging Assessment of Evolving Focal Cerebral Ischemia
Comparison With Histopathology in Rats

R.A. Knight, PhD; M.O. Dereski, PhD; J.A. Helpern, PhD; R.J. Ordidge, PhD; M. Chopp, PhD

Background and Purpose
This study was performed to document the progression of ischemic brain damage after middle cerebral artery occlusion in the rat using magnetic resonance imaging and histopathologic methods.

Methods
Cerebral ischemia was induced through permanent tandem occlusion of ipsilateral middle cerebral and common carotid arteries. The evolution of magnetic resonance imaging and histopathologic parameter changes was studied, both short term (1.5 to 8 hours) and long term (24 to 168 hours), in five specific brain regions within the middle cerebral artery territory.

Results
Significant changes in proton nuclear magnetic resonance spin-lattice and spin-spin relaxation times and the "apparent" diffusion coefficient of water could be detected within hours after the onset of permanent focal cerebral ischemia, whereas significant alterations in proton spin-density ratios were not apparent until approximately 48 hours. Histopathologic changes were evident within 12 hours, with a significant loss of neurons seen in the most severely damaged regions at 7 days. Diffusion-weighted imaging was the most sensitive technique for visualizing acute ischemic alterations. The water diffusion coefficient was the only magnetic resonance imaging parameter studied to indicate significant alterations within the first 4 hours after arterial occlusion in all five brain regions.

Conclusions
The degree of change for a particular magnetic resonance imaging parameter appeared to be related to the location and extent of neuronal injury, with the most dramatic changes occurring within the areas displaying the most severe histological damage. These results indicate that complete specification of all brain regions affected by ischemic brain injury may require a combination of imaging strategies applied over a period of days and suggest the possibility of using magnetic resonance imaging to distinguish between permanent and reversible cell damage. (Stroke. 1994;25:1252-1262.)

Key Words: cerebral ischemia • histology • magnetic resonance imaging • rats

The sensitivity of magnetic resonance imaging (MRI) to the amount and "state" of water in brain tissue has proven extremely useful for the study of stroke. MRI contrast can be manipulated such that variations produced in image intensity can be used to calculate specific MRI-measurable parameters. These parameters are extremely susceptible to changes in the biophysical environment of water and therefore may be useful for evaluating the physiological status of ischemic brain tissue. Numerous studies have reported quantitative changes in MRI parameters at various time points after experimental cerebral ischemia; however, only a small number investigate the relation of MRI parameters to the histological status of the tissue. Few studies have documented the temporal evolution of these parameters.

This study investigates the temporal relation of MRI to histopathologic events in a well-documented model of focal cerebral ischemia. MRI measurements included proton spin density ($\rho$), spin-lattice ($T_1$) and spin-spin ($T_2$) relaxation times, and the "apparent" diffusion coefficient of water ($D_w$). Regional neuronal grading and neuronal counts were used as histopathologic measures of ischemic brain damage. Preliminary results of this study on MRI data from five of the animals presented here, along with preliminary histological data, have been reported. The current study presents data from a total of 34 animals and includes regional neuronal counts, with the number of brain regions studied enlarged to encompass the entire middle cerebral artery (MCA) territory, and hemispheric neuronal counts.

The data obtained in this study demonstrated varying degrees of ischemic brain damage, measured both histologically and using MRI techniques, among the five brain regions studied. Diffusion-weighted imaging (DWI) proved to be the most sensitive MRI technique used for the detection of early ischemic damage, with calculated $D_w$ values being significantly reduced in all brain regions studied within the ischemic hemisphere by 4 hours after MCA occlusion. $T_1$ and $T_2$ values were also significantly elevated within 4 hours, but only in regions that histologically demonstrated severe neuronal damage. Significant changes in proton spin-density ratios were not seen until 48 hours after occlusion.

This work provides new information about the relative time course of changes in MRI parameters and their relation to the histopathologic status of ischemic brain damage.
Temporal Distribution of NMR and Histological Measurements

<table>
<thead>
<tr>
<th>Time, h</th>
<th>T&lt;sub&gt;1&lt;/sub&gt;</th>
<th>T&lt;sub&gt;2&lt;/sub&gt;</th>
<th>r&lt;sub&gt;p/w&lt;/sub&gt;</th>
<th>D&lt;sub&gt;ww&lt;/sub&gt;</th>
<th>NMR With Histopathology</th>
<th>Histopathology Without NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=4)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sham (n=4)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>0-2</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>10</td>
<td>...</td>
<td>3</td>
</tr>
<tr>
<td>2-4</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4-6</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>16</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>6-8</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>10</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>4-8</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>...</td>
<td>4</td>
<td>...</td>
</tr>
<tr>
<td>18-30</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>5</td>
<td>...</td>
</tr>
<tr>
<td>36-48</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>3</td>
<td>...</td>
</tr>
<tr>
<td>96-120</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>3</td>
<td>...</td>
</tr>
<tr>
<td>168</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>...</td>
</tr>
</tbody>
</table>

NMR indicates nuclear magnetic resonance.

Materials and Methods

Fasted male Fischer rats (n=34, 175 to 250 g) were anesthetized with a mixture of N<sub>2</sub>O (69%), O<sub>2</sub> (30%), and halothane (1.0% to 1.5%) administered through a face mask. Animals were allowed to breathe spontaneously throughout all surgical and MRI procedures. Focal cerebral ischemia was induced as previously described<sup>14</sup> by permanent tandem occlusion of the left common carotid artery and the left MCA, a method similar to that described by Brint et al.<sup>15</sup> Sham-operated rats (n=4) were prepared using the same procedure; however, the MCA remained intact. All studies were performed in accordance with institutional guidelines.

Immediately after surgery, each rat was placed in a Plexiglas animal holder/MRI probe apparatus and positioned inside the magnet. The animal's head was held in place inside the imaging coil by ear bars and an adjustable mouthpiece. Halothane concentration was decreased to 0.75%, and rectal temperature was maintained at 36°C to 37°C using a feedback-controlled water bath while the rat was inside the magnet. The animal's head was held in place inside the magnet.

All MRI measurements were performed using a 1.89-T, 60-cm-bore superconducting magnet (Oxford Magnet Technologies) interfaced to a Biospec I spectrometer (Bruker Instruments). Images were obtained from a 3-mm-thick coronal section using a 6-cm field of view and reconstructed using a 128 x 128 image matrix. Accurate positioning of the brain was obtained from homologous areas in the ischemic and contralateral sides of the brain were obtained, and the data are reported as the ratio of ischemic to contralateral signal intensities (p<sub>i</sub>/p<sub>c</sub>).

A measure of the relative proton (H) spin density (p) was obtained from the first image (TE, 10 milliseconds; TR, 3.0 seconds) of the T<sub>1</sub>/T<sub>2</sub> series. Image-intensity measurements from homologous areas in the ischemic and contralateral sides of the brain were obtained, and the data are reported as the ratio of ischemic to contralateral signal intensities (p<sub>i</sub>/p<sub>c</sub>).

The H water diffusion coefficient, D<sub>ww</sub>, was measured using the pulsed gradient spin-echo MRI method described by LeBihan et al.<sup>17,18</sup> Diffusion-weighted 2DFT spin-echo images (TR, 1.5 seconds; TE, 130 milliseconds) were obtained using gradient b values of 0, 292, 659, 1179, and 1825 s/mm<sup>2</sup>. Each image was signal averaged twice to improve the signal-to-noise (S/N) ratio. D<sub>ww</sub> measurements were obtained from ROIs identical to those used for T<sub>1</sub>, T<sub>2</sub>, and proton density measurements.

The brains of 26 of the MRI study animals were also taken for histopathologic examination. Rats studied using MRI were killed at various time points ranging from 8 hours to 20 days after MCA occlusion. Immediately after the final MRI session, both MCA-occluded and sham-operated rats were killed via vascular washout with heparinized saline followed by intracardiac perfusion with 4% buffered formaldehyde. The number of rats killed at each time point is shown in the Table. The animals used in these experiments were also part of a larger study that provides a more detailed histopathologic analysis from a total of 62 animals for time points ranging from immediately after MCA occlusion to 30 days after occlusion.<sup>19,20</sup> Histological data presented for time points less than the initial surgical and imaging procedure (1.5 to 8 hours after MCA occlusion), all long-term study animals were allowed to recover and were given free access to food and water for the duration of the experiment. They were reanesthetized for sequential measurements at 1, 2, 4, and 7 days after MCA occlusion.

A detailed description of the MRI methodology used in these experiments has been presented previously.<sup>21</sup> Briefly, T<sub>1</sub> values were measured using a saturation recovery two-dimensional Fourier transform (2DFT) spin-echo MRI experiment with an incremented repetition delay (repetition time [TR] of 0.75, 1.0, 1.5, and 3.0 seconds) and a fixed time-to-echo (echo time [TE], 10 milliseconds). T<sub>2</sub> values were measured using a 2DFT MRI experiment with an incremented time-to-echo (TE) of 10, 29, 59, 109, and 209 milliseconds) and a fixed repetition time (TR, 3.0 seconds). Regions of interest (ROI) were analyzed by measuring the average pixel intensity value for a given ROI from each image in the series. Image-intensity data were obtained from the same ROIs at each time point the rat was studied.

A measure of the relative proton (H) spin density (p) was obtained from the first image (TE, 10 milliseconds; TR, 3 seconds) of the T<sub>1</sub>/T<sub>2</sub> series. Image-intensity measurements from homologous areas in the ischemic and contralateral sides of the brain were obtained, and the data are reported as the ratio of ischemic to contralateral signal intensities (p<sub>i</sub>/p<sub>c</sub>).

Image-intensity measurements were obtained using a 2DFT MRI experiment with an incremented time-to-echo (TE) of 10, 29, 59, 109, and 209 milliseconds) and a fixed repetition time (TR, 3.0 seconds). Regions of interest (ROI) were analyzed by measuring the average pixel intensity value for a given ROI from each image in the series. Image-intensity data were obtained from the same ROIs at each time point the rat was studied.

Histological data presented for time points less than the initial surgical and imaging procedure (1.5 to 8 hours after MCA occlusion), all long-term study animals were allowed to recover and were given free access to food and water for the duration of the experiment. They were reanesthetized for sequential measurements at 1, 2, 4, and 7 days after MCA occlusion.
8 hours after MCA occlusion were obtained from additional animals that were not studied using MRI. No difference in histological measurements was found between animals studied using both histology and MRI and those studied with histological measures alone.

All brains were removed 24 hours after death and placed in fixative for 1 week. The brains were cut into 3-mm-thick coronal slices for histopathologic processing and embedded in paraffin. Sections (6-μm) were taken at a level corresponding to that of the MRI slice. One section was stained with hematoxylin and eosin for neuronal grading of ischemic damage and neuronal counts. An adjacent section was stained using a naphthol AS-D chloroacetate esterase method for neutrophil detection. Histological evaluation consisted of neuronal grading and neuronal counts for regions 1 through 5 and hemispheric neutrophil counts as a function of time. The histological grading scale used in this study, previously described by Dereski et al., was developed to account for both the severity and the temporal evolution of the ischemic damage. It ranges from 0 to 12, with 0 representing no damage and increasing severity of injury indicated by elevated scores. Total neuronal counts, consisting of all neurons with recognizable nuclei, were taken from a 0.5-mm² field within each of the specified brain regions. Hemispheric neutrophil counts were obtained from both the MCA-occluded and contralateral hemispheres.

ROIs were specified based on anatomically defined zones used for histopathologic analysis of ischemic damage (Fig 1). Care was taken to ensure that MRI and histopathologic data were obtained from similar regions, although exact regional correlations were impossible. The region directly under the craniectomy exhibited large magnetic susceptibility effects and was not used for MRI measurements. The size of the ROI was adjusted so as to obtain information from as many pixels as possible, without extending beyond the perimeter of the defined area (4×4 pixels: regions 1 and 3; 2×4 pixels: regions 2, 4, and 5). Similar ROIs were also defined for the contralateral hemisphere. Using the sagittal sinus and the midline of the brain as reference points, measurements from identical ROIs were obtained for each animal at each time point studied.

MRI data obtained from rats before MCA occlusion were compared to measurements from sham-operated rats using a paired t test. No significant differences were detected between normal and sham-operated rats in any brain region; therefore, the MRI data were combined to produce a control group (n=8, data shown from time=0 hours) for comparison with postischemia measurements. MRI measurements of T1, T2, and D were analyzed for statistical significance in two ways: (I) by comparing the control group measurements to postisch-
emic measurements using a two-sample t test and (2) by comparing measurements obtained from normal and ischemic-hemisphere ROIs for each time interval using paired t tests. Proton density measurements were analyzed using a two-sample t test to compare control group and postischemic values. Due to the multiple testing involved in this analysis, a critical P value (P<.01) was used to indicate significance. Analysis of histological data was performed by comparing measurements obtained immediately after MCA occlusion to those obtained at all other time points. Conservative levels of significance (P<.01) also were used for neuronal grading, neuronal counts, and neutrophil counts. Correlations of neuronal grading and neuronal counts with changes in $D_s$, $T_1$, $T_2$, and $\rho/\rho_n$ at 8, 24, and 168 hours were estimated using Pearson's correlation coefficient. Because of the large number of analyses performed on a small number of animals, correlative data are considered suggestive rather than highly conclusive.

Results

Figs 2 through 6 show neuronal scoring, neuronal counts, and MRI measurements obtained for regions 1 through 5, respectively, at specific time points after MCA occlusion. Neuronal grading of ischemic brain damage ranged from mild injury (region 5), which appeared to improve over the 7-day study period, to severe irreversible damage (region 1), which progressed to complete necrosis and eventual cavitation. Sham-operated rats killed at 24 hours showed sparse histological damage directly beneath the craniectomy site, which was readily distinguishable from that seen in MCA-occluded animals killed at the same time point. In contrast, neuronal counts showed that regions 1 and 2 were the only areas to show a significant loss of neurons at any time after MCA occlusion. No significant correlations were found between histopathologic measures and MRI parameters.

In region 1 (Fig 2), ischemic damage was apparent shortly after occlusion of the MCA. Neuronal grading of ischemic damage showed a slight increase during the initial 8 hours, followed by a dramatic increase between 8 and 24 hours after occlusion, which corresponded to the appearance of eosinophilic neurons and persisted for the duration of the study. A significant decline in neuronal counts was observed at 168 hours, with a precipitous drop occurring between 96 and 168 hours. The region progressed to complete necrosis with eventual cavitation. A significant decrease in $D_s$ was observed between 1.5 to 8 hours, which resolved to normal values at 24 and 48 hours and then became significantly elevated at 96 and 168 hours. The region demonstrated similar temporal profiles, with both parameters showing a progressive increase up to 24 hours after occlusion followed by declining values between 48 and 168 hours. The value of $\rho/\rho_n$ was significantly elevated at 48 and 96 hours after MCA occlusion. $T_1$ and $T_2$ values in region 1 demonstrated similar temporal profiles, with maximal changes noted at 24 hours.

The histological grading profile of ischemic damage in region 2 (Fig 3) was similar but less severe than that seen in region 1, showing an initial response between 1.5 and 8 hours followed by the emergence of eosinophilic neurons from 8 to 24 hours after occlusion. Varying degrees of neuronal damage were seen in this region between 24 and 168 hours, with partial necrosis evident at 1 week. Neuronal scores in region 2 also demonstrated larger standard deviations than region 1, reflect-
Neuronal counts in region 2 were significantly diminished at 96 and 168 hours. A significant decrease in the $D_w$ value was detected at 1.5 hours after MCA occlusion. $D_w$ remained depressed until 24 hours, then returned to normal values between 48 and 168 hours. Unlike region 1, $D_w$ for region 2 did not increase above normal values at 96 and 168 hours. Proton density ratios in region 2 demonstrated significant increases at 48 and 96 hours after MCA occlusion. $T_1$ and $T_2$ values in this region showed a short-term temporal profile similar to that seen in region 1, with $T_1$ and $T_2$ increasing steadily between 1.5 and 24 hours. Between 24 and 48 hours, $T_1$ and $T_2$ values for region 2 remained relatively constant, then declined toward normal values at 96 and 168 hours. $T_1$ and $T_2$ values were significantly elevated at all time points studied.

Region 3 (Fig 4) exhibited moderate neuronal damage. Neuronal grading reached a maximum value between 24 and 48 hours after occlusion and then appeared to improve, with the histological score declining at 96 and 168 hours after occlusion. No significant changes in neuronal counts were observed. $D_w$ values in region 3 showed a delayed decline relative to regions 1 and 2. Significantly lower $D_w$ values, compared with those from a homologous region from the contralateral side of the brain, were not observed until 4 hours after MCA occlusion. $D_w$ remained significantly depressed between 8 and 48 hours before returning toward normal values at 96 and 168 hours after occlusion. No significant changes in $D_w$, compared with control group values, were detected in this region. The proton density value in region 3 showed little variation until 48 hours after MCA occlusion, at which time a significant elevation in $p_t/p_n$ was detected. The value of $p_t/p_n$ then returned to normal values at 96 and 168 hours. $T_1$ and $T_2$ values also showed little change except at the 48-hour time point, when $T_1$ values were significantly elevated compared with control group values, and $T_2$ values were significantly higher than those measured from a homologous region in the contralateral side of the brain.

Results shown in Fig 5 indicate that histological grading in region 4 was similar to that seen in region 3, with mild damage noted between 2 and 8 hours. The histological score worsened at 24 and 48 hours, then appeared to improve at 96 hours before worsening again at 168 hours. This late development of increased histological scoring may indicate some type of delayed neuronal damage occurring within region 4, although a decrease in neuronal counts was not observed. The short-term profile of $D_w$ values in region 4 was similar to that seen in region 1, with significantly decreased $D_w$ values detected between 1.5 and 8 hours and a return to normal at 24 hours after MCA occlusion. Significant elevation of $D_w$ was seen at 96 hours, with marginal ($P<.05$) elevation at 48 hours, for both ischemic versus contralateral and control group versus postischemic measurements. No differences in $D_w$ were detected at 168 hours. $T_1$ and $T_2$ values in region 4 were similar to those seen in regions 1 and 2, being significantly elevated at all time points except 168 hours. At 168 hours, only $T_1$ remained significantly elevated compared with the contralateral region. Of particular note is the observation that the absolute values of $T_1$ and $T_2$ at the 24- and 48-hour time points in region 4 were higher than those found in any of the other regions at these times. No significant differences in the proton density ratio in region 4 were detected at any of the time points studied, although marginal significance ($P=.02$) was indicated at 48 hours.
Results for region 5 are shown in Fig 6. Neuronal scoring indicated only mild damage in this region, the worst score appearing at 48 hours, and no change was observed in neuronal counts. Again, the highest histological score was associated with a large standard deviation. Measurement of $D_w$ showed a significant decline, compared with the contralateral brain region, between 1.5 and 6 hours. The depressed $D_w$ value in this region resolved between 8 and 96 hours, then worsened again, becoming significantly lower than measurements from the contralateral brain region, at 168 hours after MCA occlusion. Measurements of $T_1$, $T_2$, and $\rho/\rho_n$ failed to
Neuronal grading data show that diffusion-weighted MRI was the only parameter to correctly identify all brain tissue regions that were, to some degree, affected by the ischemic insult. Visual comparison of DWI or possibly a selective vulnerability of cells in these regions. The most profound histological changes occurred in the central core of the ischemic lesion where neuronal grading data were observed in regions 2, 3, 4, 7, and 5, with the overall variability of tissue damage most pronounced in the region of damage identified histologically. 

The data obtained in this study indicate a significant reduction in \( D_w \), in ischemic brain, which represents a reduction in the translational freedom of the water molecules, precedes changes in any other MRI-measurable parameter studied to date. The exception of the diffusion coefficient, contrast-enhanced gradient-echo MRI techniques, has not been significant in our study as well. Our data support these findings, indicating a significant correlation between MRI and histopathologic parameters. In contrast to our findings, Minematsu et al. reported positive correlations, after MCA occlusion in the rat, between percentage of hemispheric lesion area at 24 hours after insult and DWI signal-intensity ratios obtained both before and after reperfusion. The lack of significant correlations in our study could be due to differences in experimental methodology, methods of data analysis, or small sample size for histological assessment.

The decline of \( D_w \) in ischemic brain, which represents a reduction in the translational freedom of the water molecules, precedes changes in any other MRI-measurable parameter studied to date. The exception of the diffusion coefficient, contrast-enhanced gradient-echo MRI techniques, has not been significant in our study. Our data support these findings, indicating a significant correlation between MRI and histopathologic parameters. In contrast to our findings, Minematsu et al. reported positive correlations, after MCA occlusion in the rat, between percentage of hemispheric lesion area at 24 hours after insult and DWI signal-intensity ratios obtained both before and after reperfusion. The lack of significant correlations in our study could be due to differences in experimental methodology, methods of data analysis, or small sample size for histological assessment.

The decline of \( D_w \) in ischemic brain, which represents a reduction in the translational freedom of the water molecules, precedes changes in any other MRI-measurable parameter studied to date. The exception of the diffusion coefficient, contrast-enhanced gradient-echo MRI techniques, has not been significant in our study. Our data support these findings, indicating a significant correlation between MRI and histopathologic parameters. In contrast to our findings, Minematsu et al. reported positive correlations, after MCA occlusion in the rat, between percentage of hemispheric lesion area at 24 hours after insult and DWI signal-intensity ratios obtained both before and after reperfusion. The lack of significant correlations in our study could be due to differences in experimental methodology, methods of data analysis, or small sample size for histological assessment.

The decline of \( D_w \) in ischemic brain, which represents a reduction in the translational freedom of the water molecules, precedes changes in any other MRI-measurable parameter studied to date. The exception of the diffusion coefficient, contrast-enhanced gradient-echo MRI techniques, has not been significant in our study. Our data support these findings, indicating a significant correlation between MRI and histopathologic parameters. In contrast to our findings, Minematsu et al. reported positive correlations, after MCA occlusion in the rat, between percentage of hemispheric lesion area at 24 hours after insult and DWI signal-intensity ratios obtained both before and after reperfusion. The lack of significant correlations in our study could be due to differences in experimental methodology, methods of data analysis, or small sample size for histological assessment.
regions, to approximately 50% of control group values, at the earliest time point studied. Additionally, region 3 of the neocortex, which was only mildly affected by the ischemic event, shows a delay in both the decline and return of $D_w$. This finding suggests that the decrease in $D_w$ may not occur as rapidly in mildly damaged cortical tissue as in more heavily damaged regions, which may be related to differences in collateral blood flow. It is particularly interesting that in heavily damaged regions the value of $D_w$ returns toward normal values at the point where large numbers of eosinophilic neurons begin to emerge. Regions 1 and 4 actually show an elevation in $D_w$ at later times; therefore, the return of $D_w$ may indicate the loss of cell membrane integrity (ie, allowing less restricted movement of the water molecules) and/or an increase in the extracellular space (ie, on the basis of the cytotoxic edema model where the extracellular $D_w$ is assumed to be much higher than that of the intracellular water) possibly signifying the end of a therapeutic window. Similar behavior has been recently documented in a reperfusion model of focal cerebral ischemia in rats and also in humans, although the exact timing of the return of $D_w$ to normal values appears to differ somewhat in humans. Disparities in the time of $D_w$ return between animal and human data are most likely due to differences in collateral blood flow to ischemic brain regions or may be species dependent.

The data obtained in this study indicate an independent behavior of $T_1$, $T_2$, and proton density from $D_w$, since these parameters remain elevated while $D_w$ returns to normal or elevated levels. Our findings are supported by another study that showed that $D_w$ returns to normal (or slightly above) within 10 minutes after reperfusion, although $T_2$ values continued to increase.

These combined findings demonstrate a clear dissociation of changes in $D_w$ and $T_2$.

The hyperintense appearance of ischemic damage in DWI obtained in this study, however, cannot be attributed solely to decreased $D_w$ values. Because relatively short TRs and long TE are generally used, DWI is also affected by $T_1$, $T_2$, and proton density contrast. Because of these contributions to DWI intensities, particular caution must be exercised when drawing conclusions from the appearance of DWI or signal-intensity ratios obtained from DWI. The data obtained in this study, when combined with those of other investigators who report changes in DWI at times less than 1.5 hours postischemia, indicate that the hyperintensity observed in DWI of cerebral ischemia is least affected by proton relaxation during the extremely acute phase (ie, <1 hour) of ischemia. At later times the effect of $T_1$ and $T_2$ relaxation becomes progressively more important in determining the appearance of DWI. This effect can be seen from the DWI obtained in this study at 48 hours after MCA occlusion (Fig 9), where a hyperintensity in the ischemic region is visible even though $D_w$ values in all brain regions except region 3 would have returned to approximately normal values.

Other findings of this study are related to changes in $T_1$, $T_2$, and $\rho_1/\rho_w$. Proton density measurements from regions 1 through 4 all demonstrated peak values at 48 hours after MCA occlusion. Since the majority of mobile protons that contribute to the MRI signal exist in the form of water, hyperintensities observed in proton density images are attributed to increased tissue water content. However, increased tissue water content in stroke, as measured by MRI, can occur by either direct movement of water into ischemic cells from the extracellular space (cytotoxic) and blood vessels (vasogenic) via osmotic gradients or may be caused by a shift in the exchange rate of protons between mobile and immobile proton pools, such that more mobile protons are available to contribute to the MRI signal but the total water content remains the same. Additionally, proton relaxation in biological tissue is highly sensitive to the structure of water near the surface of macromolecules, thus exchange between these two proton pools is believed to have profound effects on water proton relaxation rates.

MRI diagnosis of clinical stroke is usually performed using techniques that generate $T_1$ and $T_2$ contrast. This study indicates that $T_1$ and $T_2$ values in the most heavily damaged regions (regions 1, 2, and 4) show a similar temporal response, with values increasing progressively during the first 24 hours after MCA occlusion, then declining toward normal values during the remainder of the experiment. These findings are consistent with previous studies that have reported significant elevations of $^1$H $T_1$ and $T_2$ relaxation times in stroke-affected brain tissue in both experimental animals and humans. The results of this study suggest that $T_1$- and $T_2$-weighted MRI methods are most effective more than 24 hours after stroke, whereas detection using proton density-weighted images was best more than 48 hours after stroke.

If the increased relaxation times observed in stroke were due entirely to edema, then one would expect that proton density values would peak at the same time. Our findings, however, indicate that proton density ratios in regions 1 through 4 reach maximum values at 48 hours after MCA occlusion; therefore, increased tissue water content alone cannot account for the elevated relaxation times observed at 24 hours. One explanation is that changes in relaxation observed after cerebral ischemia are also related to changes in the ratio of free to bound water. This hypothesis is supported by magnetization transfer contrast MRI studies of cerebral ischemia in rats reported by Ordidge et al., which show a reduction in the exchange rate between free and bound protons at 24 hours. Region 3 demonstrated moderate histological damage, which appeared to im-
prove with time, and delayed response of $T_1$ and $T_2$ until 48 hours when $\rho/\rho_0$ was elevated. This delay may indicate that increased $T_1$ and $T_2$ values are caused primarily by edema in brain regions where the cells have been affected by ischemia but are capable of recovery. A shift in bound-to-free water equilibrium also may be a contributing factor in more heavily damaged areas, thus producing a more dramatic increase in $T_1$ and $T_2$. A related analysis of $T_1$ and $T_2$ MRI data from 10 of the rats used in this study, performed using a linear-filter method to suppress undesirable interfering image features, revealed the evolution of two distinctly different zones based on $T_1$ and $T_2$ relaxation.

It is interesting that region 4 demonstrated the largest increase in relaxation times of the five brain regions studied, although histological damage was only moderate. The exaggerated increase in $T_1$ and $T_2$ in region 4 is difficult to explain, but we speculate that it may be related to differences in cell types. Regions 4 and 5 are composed of a mixture of both gray and white matter, whereas regions 1, 2, and 3 are cortical, containing only gray matter. Others have suggested that myelin and myelin-breakdown products may be significant factors in the determination of relaxation rates for white matter in normal and stroke-affected brain tissue.\textsuperscript{37,38}

The contribution of neutrophil infiltration to ischemic damage may also be significant; however, the relation of activated neutrophils to changes in MRI parameters after cerebral ischemia has not been reported previously. The data obtained in this study indicate that significant numbers of neutrophils were present in the ischemic hemisphere at 48 and 72 hours after MCA occlusion, corresponding to the time when eosinophilic neurons began to appear (ie, indicative of irreversible neuronal damage). Neutrophils migrate to an area of inflammation in response to the release of chemotactic substances.\textsuperscript{42-46} Once activated, they adhere to the endothelium, where they can cause direct endothelial damage, then cross over into the extravascular space.\textsuperscript{47} Neutrophils can initiate rapid degeneration of the extracellular matrix molecules, causing focal cellular damage and enhancing membrane leakage,\textsuperscript{48} and may also compromise tissue perfusion.\textsuperscript{49,50} A loss of cell membrane integrity due to neutrophils may play a role in the return of $D_2$ to normal values and the subsequent rise seen in more severely damaged brain regions.

MRI shows exceptional promise for investigating the mechanisms responsible for ischemic injury and the eventual outcome of stroke. The findings of this study show a relation between MRI and histological parameters and indicate that MRI may be useful for investigating the histological status of ischemic brain tissue and monitoring of certain therapeutic regimens. Further studies may lead to an increased understanding of ischemic damage and possibly provide a method for assessing the status of stroke-induced brain damage in humans.

Acknowledgments

This study was supported in part by National Institutes of Health grants NS-23393 and NS-29463. We are grateful to L.C. Copeland for her assistance with the surgical preparation and Z.X. Qing and A.M.Q. Vande Linde for their assistance with the MRI data analysis.

References


Editorial Comment

Diffusion-weighted imaging (DWI), the latest advance in magnetic resonance (MR) methodologies, is beginning to add to our understanding of the pathophysiology of stroke. Using diffusion of water as the source for images, DWI detects changes in water diffusion rates that occur very early in the course of tissue damage. The importance of this new modality lies in its ability to show ischemic injury before it is demonstrated by T1-weighted MR imaging. The major drawback has been the issue of the significance of these early changes and their biophysical basis.

The article by Knight et al in this issue addresses the question of significance. By carefully comparing the DWI with subsequent histological changes, the authors clearly demonstrate that the observed changes in water movement occur in regions with injured neurons that are destined to die. Although DWI did not always correlate with histology, particularly in the periphery of the lesions, the correlations were very good in most areas of injury.

In another recent study, loss of ATP and fall in pH were observed in regions showing changes on DWI. Lactate changes, however, were more widespread than those seen on DWI, and there was a dissociation between lactate and pH around the lesions. The changes in water contributing to the diffusion changes are unclear. Water behaves in a complex manner in biological tissues. Ischemia further complicates the picture. Cell swelling caused by cytotoxic edema reduces the extracellular space, which could restrict water movement. The authors suggest that there is a change in free-to-bound water, which could be brought about by hypoxia. The return of the diffusion images to normal at a time when the T1- and T2-weighted images are abnormal remains to be ex-
Magnetic resonance imaging assessment of evolving focal cerebral ischemia. Comparison with histopathology in rats.
R A Knight, M O Dereski, J A Helpern, R J Ordidge and M Chopp

Stroke. 1994;25:1252-1261
doi: 10.1161/01.STR.25.6.1252

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/25/6/1252