Correlation of Cerebral Hypoxic-Ischemic T2 Changes With Tissue Alterations in Water Content and Protein Extravasation

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Background and Purpose—Age-dependent changes in T2-weighted MR images have been reported in cerebral hypoxia-ischemia. However, the biophysical mechanisms responsible for the image changes remain poorly defined. We investigated whether cerebral hypoxia-ischemia–induced T2 changes correlate with alterations in either water content or protein extravasation.

Methods—One- and 4-week-old rats were subjected to unilateral carotid artery occlusion plus hypoxia in 8% oxygen. T2 images were acquired before, during, and 1 or 24 hours after hypoxia-ischemia. Blood-brain barrier disruption and brain edema were evaluated by immunohistological detection of IgG extravasation and measurement of water content by dry-wet weight and specific gravity methods.

Results—In 1-week-old rats, T2 values, areas of hyperintensity on T2-weighted images, and water content in the ipsilateral hemisphere increased during hypoxia-ischemia, recovered at 1 hour after hypoxia-ischemia, and increased again at 24 hours after hypoxia-ischemia. Extravasation of IgG occurred during hypoxia-ischemia and remained detectable 24 hours after hypoxia-ischemia. In 4-week-old rats, an increase in T2 or extravasation of IgG did not occur until 24 hours after hypoxia-ischemia despite a comparable elevation in water content during and soon after hypoxia-ischemia.

Conclusions—T2 imaging appears reliable for detecting edema associated with disruption of the blood-brain barrier but not necessarily an increase in cerebral water or plasma proteins alone. The different hypoxic-ischemic changes in T2 in immature and older brain are associated with differences in alterations in water content plus extravasation of protein, consistent with age-dependent differences in hypoxic-ischemic alterations in vascular permeability. (Stroke. 2001;32:958-963.)

Key Words: blood-brain barrier • brain edema • cerebral ischemia • hypoxia • magnetic resonance imaging

T2-weighted MR images receive their contrast from the transverse magnetic relaxation of water protons in tissue. This type of MRI has been widely used to detect ischemic changes in cerebral tissue.1–5 Hyperintense changes in T2-weighted images and increases in T2 (the transverse relaxation time) start to evolve 2 to 3 hours after an ischemic insult in 4-week-old and adult rats, and these changes have been believed to be related to the presence of a vasogenic edema.3,4,6,7 By 24 to 48 hours, the areas of hyperintensity generally correspond well to the size of the infarct determined by histological techniques.5,9 In contrast, an elevation in T2 occurs as early as 30 minutes after the onset of hypoxia-ischemia in neonatal or immature brain, differing from the delayed response reported in 4-week-old and adult rats.4,5 We speculated that during hypoxia-ischemia there are age-dependent differences in brain water produced by hypoxia-ischemia. Note that several hours after an episode of cerebral ischemia, a vasogenic edema develops that is associated with opening of the blood-brain barrier (BBB) and leakage of large molecules, such as plasma proteins, in addition to water from the systemic circulation into the parenchyma.10,11 However, despite changes in T2 being linked with such a vasogenic edema, relatively few studies have examined directly the correlation between T2, tissue water content, and protein extravasation after an ischemic insult.

In the present study we hypothesized that the different MR responses to hypoxia-ischemia in 1- and 4-week-old brain are related to age-dependent differences in ischemic changes in brain water and vascular permeability. To test this, we measured the temporal and spatial changes in T2 and brain water content and assessed BBB disruption during and after cerebral hypoxia-ischemia in 1- and 4-week-old rats. The maturity of the rat brain at these ages corresponds roughly to newborn and juvenile (prepuberty) stages of human development, respectively.12–14
Materials and Methods

Model of Hypoxia-Ischemia

Pregnant Wistar rats were obtained from Charles River Laboratories (Montreal, Canada) and gave birth approximately 1 week after their arrival, after which the litter was culled to 9 to 10 pups. All animals were treated in accordance with the guidelines provided by the Canadian Council on Animal Care, and experiments were approved by the local Animal Care Committee. Animals were assigned to 2 age groups: 1- and 4-week-old rats. Cerebral hypoxia-ischemia was produced as described previously. Briefly, the right common carotid artery was ligated and severed under isoflurane (2.5%) anesthesia. The incision site was closed, and saline (0.1 mL/10 g IP) was injected to compensate for any fluid losses during surgery. In the sham control group, the carotid artery was isolated but not ligated. After surgery, the rats were returned to the cage with the mother for 1 to 2 hours of recovery from the anesthesia. Rats were then exposed to hypoxia by spontaneous breathing of humidified 8% O2 in 92% nitrogen for a duration of 2 hours for 1-week-old rats or 30 minutes for 4-week-old rats, thereby producing a cerebral infarct of similar size. Body temperature was maintained at 37.0°C to 37.5°C during hypoxia with a heating lamp and circulating water blanket.

Magnetic Resonance Imaging

T2-multiecho imaging was performed in a 9.4-T/21-cm horizontal bore magnet (Magnex) equipped with an MSLEX Bruker console (Bruker). The animals were anesthetized with isoflurane (0.5% to 1.25%) and placed in a chamber designed to fit the bore of the magnet. In 1-week-old rats, the head was restrained with a foamed ear pin, and an incisor bar within a quadrature coil was tuned to 400.045 MHz. Respiration rate was measured by a small plastic tube placed inside the nose. In 1-week-old rats, the head was restrained with ear pins, and an incisor bar within a quadrature coil was tuned to 400.045 MHz. Respiration rate was monitored continuously while in the magnet. Rats (n = 5 for each age group) were imaged immediately before hypoxia-ischemia, during hypoxia-ischemia, and at 1 hour or at 24 hours after hypoxia-ischemia. T2-multiecho images were acquired with a spin-echo sequence with the following parameters: repetition time = 1200 ms, echo time = 21.6 ms, 6 echo images, and 3 slices at a thickness of 1 mm for 1-week-old and 1.5 mm for 4-week-old rats. The field of view was 2 cm, and the object matrix was 256 x 128. Immediately after the last image, the animals were injected with pentobarbital (80 mg/kg), and an incisor bar within a quadrature coil was tuned to 400.045 MHz. Respiration rate was monitored continuously while in the magnet. Rats (n = 6 for each age group) were imaged immediately before hypoxia-ischemia, during hypoxia-ischemia, and at 1 hour or at 24 hours after hypoxia-ischemia. T2-multiecho images were acquired with a spin-echo sequence with the following parameters: repetition time = 1200 ms, echo time = 21.6 ms, 6 echo images, and 3 slices at a thickness of 1 mm for 1-week-old and 1.5 mm for 4-week-old rats. The field of view was 2 cm, and the object matrix was 256 x 128. Immediately after the last image, the animals were injected with pentobarbital (80 mg/kg), and the brain was removed and processed for the assessment of brain water as described below. The T2 relaxation times in the ipsilateral or contralateral hemisphere at the level of striatum and posterior thalamus were measured from an analysis of the multiecho images with image analysis software. Areas of T2 hyperintensity were determined from the T2-multiecho images at the level of the thalamus, where the intensity levels in the contralateral cortex were used to define a threshold intensity, and the software then allowed the determination of the area of the pixels exceeding this threshold. These areas were converted to a percentage of the entire brain slice.

Assessment of Changes in Tissue Brain Water

Changes in brain water or specific gravity were assessed in 1-week-old (n = 38) and 4-week-old (n = 33) rats; subgroups of animals that were killed at 1 hour and 24 hours after hypoxia-ischemia also had MRI. There were no differences in water content, specific gravity, or their changes during hypoxia-ischemia between animals with MRI and those without MRI; therefore, these data were combined. Rats were decapitated either during hypoxia-ischemia (n = 9 for 1-week-old rats, n = 16 for 4-week-old rats) or 1 hour after 1-week-old rats, n = 11 for 4-week-old rats) or 24 hours (n = 7 for 1-week-old rats, n = 6 for 4-week-old rats) after hypoxia-ischemia. Animals with sham surgery but not hypoxia-ischemia served as a control (n = 6 for each age group). The whole cerebrum was removed and dissected into 3 parts. The anterior cerebrum, including the striatum, was removed for the immediate measurement of brain water content. The middle cerebrum was frozen in isopentane (−45°C) and stored at −80°C for future examination of disruption of the BBB. The posterior cerebrum was removed for the immediate determination of tissue specific gravity. A dry/wet weight method was used to measure brain water content in samples of left and right anterior cerebrum consisting of approximately 50 to 60 mg of tissue for 1-week-old rats or 100 to 120 mg of tissue for 4-week-old rats. The tissue sample was wrapped in a piece of preweighed aluminum foil, reweighed, and dried in an oven at 100°C for 4 to 5 days until the weight of dry tissue was constant. The water content was calculated as the difference between wet and dry weight of the sample and then converted to a percentage of its wet weight.

Brain specific gravity, which is inversely dependent on brain water content, was measured according to published methods with some modifications. A linear density gradient was prepared with a colloidal suspension of silica (Percoll; Pharmacia Biotech) and NaCl solution. Stock isotonic Percoll (1.5 mol/L Percoll mixed with 1.5 mol/L NaCl in a 9:1 ratio) was diluted to a “dense” solution with 0.15 mol/L NaCl as recommended by the manufacturer. Equal volumes of dense and “light” solution (0.15 mol/L NaCl) were pumped into a graduated cylinder, producing a linear Percoll gradient. The depth of the gradient was calibrated for specific gravity with the use of standard solutions. The yield of tissue was calculated as described below. The T2 relaxation times in the ipsilateral or contralateral hemisphere at the level of striatum and posterior thalamus were measured from an analysis of the multiecho images with image analysis software. Areas of T2 hyperintensity were determined from the T2-multiecho images at the level of the thalamus, where the intensity levels in the contralateral cortex were used to define a threshold intensity, and the software then allowed the determination of the area of the pixels exceeding this threshold. These areas were converted to a percentage of the entire brain slice.

Immunohistological Detection of IgG Extravasation

IgG extravasation was assessed in sections from the middle cerebrum of 1-week-old (n = 23) and 4-week-old (n = 29) animals killed, including sham controls (n = 4 for 1-week-old rats, n = 6 for 4-week-old rats), during hypoxia-ischemia (n = 4 for 1-week-old rats, n = 8 for 4-week-old rats), 1 hour after hypoxia-ischemia (n = 9 for 1-week-old rats, n = 8 for 4-week-old rats), or 24 hours after hypoxia-ischemia (n = 6 for 1-week-old rats, n = 7 for 4-week-old rats). Frozen sections (20 μm thick) were fixed in acetone and mounted onto polylysine-coated slides. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in methanol followed by blocking with 10% serum. The slides were incubated at room temperature for 1 hour with donkey anti-rat IgG antibody (Jackson Immunoresearch) at dilutions of 1:200 for 1-week-old rats and 1:400 for 4-week-old rats. IgG was revealed with streptavidin–horseradish peroxidase (1:400, Dako) and diaminobenzidine. Regions of increased immunolabeling with IgG had well-defined boundaries and were readily distinguished from regions of lower labeling in adjacent or contralateral regions. Areas of increased IgG labeling were measured and converted to a percentage of the entire section at the level of the mid-thalamus with the use of a computerized image analysis system (MCID, Imaging Research Inc).

Statistical Analysis

Grouped data are presented as mean±SD. A comparison between means at different time points (eg, for T2 relaxation times, T2 hyperintensity areas, IgG extravasation areas, specific gravity, and water content) was performed with an ANOVA followed by a Duncan test. Differences are considered significant at P < 0.05. A least-squares regression analysis was used to analyze the correlation of T2 changes with alterations in brain water.

Results

Areas of T2 Hyperintensity and IgG Extravasation

Before hypoxia, T2-weighted images from either 1- or 4-week-old rats displayed no differences in intensities between hemispheres (Figure 1). In addition, IgG accumulation was essentially undetectable within the brains of sham control animals. The exception was two 1-week-old rats that had some IgG labeling in periventricular regions bilaterally, consistent with the high permeability of periventricular regions adjacent to the choroid plexus in immature animals.
During hypoxia-ischemia in 1-week-old animals, T2 images started to appear hyperintense in the hemisphere ipsilateral to the carotid occlusion as early as 30 to 45 minutes after the start of hypoxia-ischemia. The hyperintense areas spread within the territory of the right common carotid artery as hypoxia-ischemia continued. At the end of 2 hours of hypoxia-ischemia, a large area of the striatum, cortex, hippocampus, thalamus, and hypothalamus was brighter in the ipsilateral than the contralateral hemisphere (Figures 1 and 2). Hyperintense changes were generally restricted to the hemisphere ipsilateral to the occlusion, except for 1 animal that showed a small area of hyperintensity in the contralateral hemisphere adjacent to the dorsal midline. In addition, similar to the T2 changes, IgG immunoreactivity was observed within the cortex, hippocampus, thalamus, and hypothalamus ipsilateral to the carotid artery occlusion in 1-week-old animals at the end of hypoxia-ischemia (Figures 1 and 2). In contrast to 1-week-old animals, 4-week-old animals did not have significant hyperintense changes in their T2-weighted images or IgG extravasation within their brain sections during hypoxia-ischemia (Figures 1 and 2).

On the termination of hypoxia, the regions of hyperintensity on MR images of 1-week-old rats resolved rapidly such that at 1 hour after hypoxia-ischemia the images were similar to those acquired before hypoxia-ischemia. However, IgG labeling persisted within the ipsilateral hemisphere in 1-week-old animals at 24 hours after hypoxia-ischemia, whereas there were no areas of hyperintensity on T2-weighted images and there was no IgG extravasation. At 24 hours after hypoxia-ischemia, the T2 hyperintensity and protein persisted in 1-week-old animals within the ipsilateral cortex, striatum, hippocampus, and thalamus (Figures 1 and 2). By 24 hours after hypoxia-ischemia, areas of T2 hyperintensity and IgG extravasation were observed within regions of the ipsilateral cortex, striatum, hippocampus, and thalamus in 4-week-old animals (Figures 1 and 2).
T2 Relaxation Times and Brain Water

Before hypoxia-ischemia, there was no left-right hemispheric difference in T2 or the amount of brain water in either age group (Figure 3). There was a developmental decrease in brain water content (88.5 ± 0.1% in 1-week-old rats, 81.1 ± 0.3% in 4-week-old rats). At the end of hypoxia-ischemia, the T2 ratio of ipsilateral to contralateral values in 1-week-old animals was 7% to 9% greater than the prehypoxia values acquired from either anterior or posterior cerebrum. Corresponding to the T2 increases, the water content ratio of ipsilateral to contralateral values increased, and the specific gravity ratio showed a marked decline. In 4-week-old animals, there were no significant changes in the T2 ratios despite an increase of the ipsilateral to contralateral water content ratio in the anterior cerebrum and a corresponding decrease in specific gravity in the posterior cerebrum. Although the magnitude of the elevation in water content ratio during hypoxia was similar in 1- and 4-week-old rats, the amount of water content in the hemisphere ipsilateral to the carotid occlusion in 4-week-old brain was much lower than that in 1-week-old rats (82.2 ± 0.3% versus 88.8 ± 0.5%).

One hour after hypoxia-ischemia, there was some recovery in brain water in 1- but not 4-week-old animals. The T2, brain water, and specific gravity ipsilateral to the occlusion normalized in 1-week-old animals, resulting in ipsilateral to contralateral ratios near 1. In contrast, in 4-week-old animals, T2 in the ipsilateral hemisphere remained normal, but the water content ratio remained elevated and the specific gravity ratio remained depressed.

Twenty-four hours after hypoxia-ischemia, generally concurrent imaging and tissue changes were observed in both 1- and 4-week-old animals (Figure 3). In 1-week-old brain, T2 increased by 11% to 14% in posterior and anterior brain samples, and the water content ratio further increased and specific gravity further decreased accordingly. In 4-week-old brain, T2 relaxation times increased by 4% to 6%, and the water content ratio in the anterior brain further increased, although there appeared to be a partial recovery of the specific gravity measured in the posterior cerebrum, consistent with the hypoxic-ischemic damage being less severe posteriorly\(^1\) (Figure 3). Comparison of T2 and brain water ratios at 24 hours after hypoxia-ischemia demonstrated that there was a strong linear correlation between T2 and brain water content in both 1- and 4-week-old brain (\(P<0.02\)) (Figure 4). Although there was also a trend for T2 and specific gravity to be linearly correlated in both age groups, this did not reach statistical significance (\(P>0.08\)).

Discussion

T2-weighted imaging has been used frequently in patients and experimental animals with stroke to detect cerebral changes several hours after an ischemic insult. The increases in T2 image intensity observed in such studies have generally been interpreted as being indicative of vasogenic edema or infarction.\(^3,4,6,7\) Relatively few studies have examined the direct correlation between brain water and T2. The temporal profiles of the effects of hypoxia-ischemia on MR changes and their tissue correlates in animals at different stages of postnatal development are summarized in the Table. They have demonstrated directly that T2 imaging changes are best correlated to an increase in brain water accompanied by an increase in protein content rather than increases in brain water or protein content alone. With respect to the age dependence of the changes in T2 in hypoxia-ischemia, instead of being associated with differences in edema per se, T2 changes are likely due to a combination of differences in susceptibility of the BBB to ischemic damage and ontogenic differences in the chemical changes in the brain influencing the MR visibility of water.
A good correlation between tissue T2 and water content has been observed frequently but not universally.\textsuperscript{19–22} Important factors influencing T2 are the macromolecular environment in which the water exists in addition to the water content itself.\textsuperscript{23–27} In the present study we observed no change in T2 but acute increases in brain water without a major disruption of the BBB. This edema is similar to that reported in adult animals subjected to cerebral ischemia, in which there is an intact BBB yet an influx of Na\textsuperscript{+} and water from the blood due to failure of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase or activation of Na\textsuperscript{+}/H\textsuperscript{+} exchange.\textsuperscript{10,11} Surprising is the lack of T2 changes because an absolute increase in brain water is expected to be associated with a higher T2.\textsuperscript{23} One possibility is that the absolute effect of water on the sum of magnetization in the transverse direction in the older animals was below detectable limits considering that water content in 4-week-old brain is much lower than that in 1-week-old brain. This seems unlikely because the increase in water content during hypoxia-ischemia in the 4-week-old animals was not significantly different from that at 24 hours, at which time increases in both T2 and water were observed. A more likely difference in 4- compared with 1-week-old animals is that the cellular composition and the distribution of water changes counteract the increases normally accompanying cellular edema. Cell composition certainly differs in immature and mature brain. Water content decreases with age, whereas the amounts of proteins and lipids increase, and both of these will tend to decrease T2.\textsuperscript{23,26–28} In addition, T2 is also dependent on a weighted ratio of free to bound water so that changes in the relative amount of water bound to macromolecules during ischemia could explain some of the T2 changes. Irrespective of the explanation, T2 is not necessarily sensitive for detecting the cellular edema that can occur at acute stages of ischemic injury when the BBB is known to be intact. However, there is an excellent correlation of water content and T2 in both age groups at 24 hours after hypoxia-ischemia, when breakdown of the BBB is present, suggesting that the T2 increase is a marker of vasogenic or open barrier edema.

The present results demonstrated that in neonatal rat brain, disruption of the BBB to proteins occurs earlier after a hypoxic-ischemic insult than in more mature brain. Protein-rich fluid accumulates in 1-week-old brain immediately after the insult, and similar results have been observed in 1-week-old animals previously.\textsuperscript{29} In contrast, in adults cerebral ischemia-induced vasogenic edema associated with opening of the BBB occurs 3 to 6 hours after the insult, although an intact barrier edema can be observed earlier.\textsuperscript{11} The susceptibility of the neonatal BBB to hypoxia or ischemia may be related to the immaturity of the BBB in such young animals\textsuperscript{17,30} or to other ontogenic differences, such as a relatively lower antioxidant content in neonatal brain.\textsuperscript{31} It appears that the early onset of T2 changes is linked to an early disruption of the BBB in immature brain. Whether the recovery of the T2 changes immediately after hypoxia-ischemia in neonates is related to a recovery in BBB function is not clear because we observed a return toward normal in brain water but not in IgG immunolabeling. This can reflect a recovery of the BBB if water and excess ions such as sodium are cleared from the tissue but proteins remain because of their slower clearance rate or uptake into neurons and glia.\textsuperscript{10,11} It could also reflect a restoration of ion pump function on the termination of

### Hypoxic-Ischemic Changes in T2 Relaxation Times, Water Content, and IgG Accumulation in 1- and 4-Week-Old Rat Brain

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>During HI</th>
<th>1 h After HI</th>
<th>24 h After HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week old</td>
<td>T2</td>
<td>Increase</td>
<td>Recovery</td>
<td>Increase</td>
</tr>
<tr>
<td></td>
<td>Water†</td>
<td>Increase</td>
<td>Recovery</td>
<td>Increase</td>
</tr>
<tr>
<td></td>
<td>IgG†</td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>4 weeks old</td>
<td>T2</td>
<td>No change</td>
<td>No change</td>
<td>Increase</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>No change</td>
<td>No change</td>
<td>Increase</td>
</tr>
</tbody>
</table>

HI indicates hypoxia-ischemia.

*Changes compared with before hypoxia.
†Changes compared with sham controls.
hypoxia-ischemia, resulting in a return to normal of solutes such as sodium and water but a continued disruption of the BBB. The latter seems less likely because an open BBB has been associated with even higher sodium and brain water levels.11 Additional experiments that examine BBB permeability at various time points are needed to determine whether the BBB recovers or the edema resolves.

In summary, by investigating the relationship between changes in cerebral T2 with alterations in brain water content and vascular permeability, we have demonstrated that T2 changes best serve as an indicator of vasogenic edema associated with the disruption of the BBB rather than as an indicator of an elevation in water content or increased protein content alone. Immature brain is relatively susceptible to BBB damage in the acute stages of cerebral hypoxia-ischemia, and age-dependent differences in protein extravasation and distribution of water need to be considered when T2 imaging is used to monitor hypoxic-ischemic changes in brain.

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References
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