Different Apparent Diffusion Coefficient

Water Content Correlations of Gray and White Matter During Early Ischemia

Toshihiko Kuroiwa, MD; Tsukasa Nagaoka, MD; Masato Ueki, MD; Ichiro Yamada, MD; Naoyuki Miyasaka, MD; Hideaki Akimoto, MD

Background and Purpose—Early and accurate diagnosis of brain edema in stroke patients is essential for the selection of appropriate treatment. We examined the correlations between the changes in the apparent diffusion coefficient (ADC), regional water content, and tissue ultrastructure during early focal cerebral ischemia.

Methods—The left middle cerebral arteries of cats were occluded with an intramagnet occlusion/recirculation device. T2-weighted, diffusion-weighted, and perfusion imaging were performed repeatedly during the initial 3 hours after occlusion. The ADCs obtained from ADC maps were compared with the corresponding tissue water content values determined by gravimetry and electron microscopic water localization.

Results—ADC reduction was detected in areas of low perfusion 15 minutes after occlusion and thereafter. The water content increase correlated linearly with the ADC decreases in both the gray and white matter. However, both the water content corresponding to an ADC value and the rate of ADC change of the gray and white matter differed significantly ($P<.05$) as follows: $y = -10105x + 8533$ ($r = .86$) and $y = -6174x + 4611$ ($r = .67$), respectively, where $x$ is the water content (grams water per gram tissue) and $y$ is the ADC ($\times 10^{-6}$ mm²/s). Hydropic astrocytic swelling was seen in both structures, and in the white matter, oligodendroglial and myelinated axonal swelling and periaxonal space enlargement were observed.

Conclusions—When early ischemic edema in experimental focal cerebral ischemia is evaluated with ADC mapping, the different slopes and intercepts of the water content and ADC correlation lines for the gray and white matter, which probably reflect different ultrastructural localization of water, should be taken into account. (Stroke. 1998;29:859-865.)

Key words: brain edema ■ histology ■ magnetic resonance imaging ■ middle cerebral artery occlusion ■ cats

I
n patients with cerebral infarction, accurate diagnosis in the very early phase is essential for choosing the most appropriate treatment. Ischemic edema, which appears as early as 5 minutes after ischemia onset, has been a target for early detection of cerebral ischemia. MRI for determination of the ADC is one of the most powerful methods for detecting ischemic edema.² It enables detection of ischemic edema during the cellular edema phase, when most of the ischemic tissue damage is still potentially reversible and therefore within the therapeutic window for detection. Moreover, this method can distinguish cellular from vasogenic edema, two subtypes of edema with different pathophysiologicals requiring different treatment. However, little is known about the meaning of ADC changes in terms of ultrastructural water localization in tissue showing ischemic edema. This is partly due to the heterogeneous nature of ischemic edema, which is cellular and/or vasogenic depending on the time after ischemia onset, ischemia severity, and tissue structure (gray versus white matter). Recently, the white matter was shown to be highly vulnerable to ischemic insult with ultrastructural changes developing as early as 30 minutes after ischemia onset.³ In this study we used an intramagnet MCA occlusion/recirculation device to induce cerebral ischemia in cats, and we examined the coordination of ADC changes and ultrastructural water localization during early ischemia. We also derived equations that can be used to determine the tissue water content from the ADC values during early ischemia.

Materials and Methods

Experimental Protocol

The animal experiments were performed according to a protocol approved by the Committee on Animal Research of Tokyo Medical and Dental University. Fifteen adult cats weighing 3.5 to 4.5 kg were divided into the following three groups: (1) ischemia/MRI/water content measurement (n = 6); (2) ischemia/MRI/ultrastructure examination (n = 6), and (3) controls subjected to sham operation (n = 3). Each animal was initially anesthetized with ketamine (30 mg/kg IP...
Selected Abbreviations and Acronyms

ADC = apparent diffusion coefficient
BBB = blood-brain barrier
DWI = diffusion-weighted imaging
MCA = middle cerebral artery
ROI = region of interest

ADC and Water Content Correlation

every 2 hours), intubated, and artificially ventilated under 1% isoflurane anesthesia. Catheters were placed in the right femoral artery for blood pressure and blood gas monitoring and in the right femoral vein for injection of drugs and tracers. A rectal temperature probe was connected to a feedback-controlled water jacket covering the body of the animal. The body temperature was maintained at 37°C.

An MCA occlusion/recirculation device was implanted in each cat by a transorbital approach. The animal was placed in an experimental MRI scanner, and T2-weighted imaging, DWI, and perfusion imaging were performed before and 15, 30, 60, 120, and 180 minutes after MCA occlusion.

Shortly after the final MR image was obtained, the animal was killed under anesthesia and processed for water content measurement and histological examination.

Intramagnet MCA Occlusion/Recirculation Device

We used a modified transorbital MCA occlusion method to implant an intramagnet MCA occlusion/recirculation device to produce cerebral ischemia in the magnet with minimal optic nerve damage, intracranial pressure changes, and cerebrospinal fluid leakage and also to enable recirculation to be performed if needed. With the use of a surgical microscope, the left MCA was exposed through a small bone window (approximately 5 mm in diameter) drilled close to the optic canal, and a loop of 5-0 nylon thread was placed round it. The free ends of this thread were passed through two small holes in a small polyvinyl plate (2×3×1 mm), which was placed loosely on the body of the animal. The body temperature was maintained at 37°C.

To examine the structural and ultrastructural changes corresponding to the ADC changes (group 2), the animals were perfused transcardially with a buffered solution of 3% paraformaldehyde and 1% glutaraldehyde under pentobarbital anesthesia (50 mg/kg) shortly after the final MR image was obtained.

The brain was removed and cut sequentially into 3-mm-thick coronal blocks with a tissue slicer. The blocks were placed in silicone oil (KF-96L, ShinEtsu Chemical), a coronal block corresponding to the level of the ADC map was selected, and tissue samples weighing 10 to 20 mg were excised from the sites corresponding to ROIs in the gray and white matter. The samples were dropped into a kerosene/monobromobenzene gradient column for specific gravity measurement, and the tissue water contents were obtained from the specific gravity values according to the formula reported by Marmarou et al. A series of 80 fast low-angle shot images (repetition time, 17 ms; echo time, 5 ms; flip angle, 25°; matrix, 64×64) of a single section (10 mm anterior to the auditory meatus) was acquired to plot a time-intensity curve for the brain parenchyma. The scan time per image was 1.1 seconds, and there was no interscan delay. A bolus of 0.2 mmol/kg gadopentate dimeglumine (Schering) was injected manually over approximately 1.5 seconds through the femoral venous catheter.

The signal intensities during the transit of contrast material through the ROIs in the gray matter of the bilateral middle ectosylvian gyr on the coronal image were measured, and these data were transformed into plots of \( \Delta R_t \) versus time, according to the following formula:

\[
\Delta R_t = \frac{-\ln[(S(t)/S(0))]/TE}{T_E}
\]

where \( T_E \) is the echo time, \( S(t) \) is the image intensity at time \( t \), \( S(0) \) is the precontrast baseline signal intensity, and \( \Delta R_t \) is the change in effective transverse relaxation rate. The ratio of the peak ischemic to control tissue \( \Delta R_t \) values was calculated and used as an index of the perfusion deficit in the ischemic tissue.

Water Content and BBB Findings

Brain edema in groups 2 and 3 was assessed by gravimetry. Each animal was killed with an overdose (100 mg/kg) of pentobarbital injected intravenously, then the brain was removed rapidly and cut sequentially into 3-mm-thick coronal blocks with a tissue slicer. The blocks were placed in silicone oil (KF-96L, ShinEtsu Chemical), a coronal block corresponding to the level of the ADC map was selected, and tissue samples weighing 10 to 20 mg were excised from the sites corresponding to ROIs in the gray and white matter. The samples were dropped into a kerosene/monobromobenzene gradient column for specific gravity measurement, and the tissue water contents were obtained from the specific gravity values according to the formula reported by Marmarou et al. The tissue water content determined by gravimetry was compared with the ADC value for the same ROI of the coronal slice taken from the same level. BBB permeability to serum macromolecules was assessed by examining leakage of Evans blue dye (2% wt/vol Evans blue in isotonic saline, 2 mL/kg), which was injected intravenously shortly after MCA occlusion.

Histological Examination

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The brain was removed and cut sequentially into 3-mm-thick coronal sections and placed in cooled fixative. A block corresponding to the ADC map was chosen and sampled from the sites in the gray matter and white matter of the left cingulate, middle suprasylvian, middle ectosylvian, and posterior Sylvian gyri corresponding to the ROIs for electron microscopic examination. The mirror surface coronal block was then prepared and stained with hematoxylin and eosin and cresyl violet for light microscopic examination.

| Table 1: Results of Water Content and BBB Findings

<table>
<thead>
<tr>
<th>Group</th>
<th>Water Content (%)</th>
<th>BBB Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86.0</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>88.4</td>
<td>1+</td>
</tr>
<tr>
<td>3</td>
<td>90.2</td>
<td>2+</td>
</tr>
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TABLE 1. Physiological Parameters Before and After MCA Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Before Ischemia</th>
<th>1 h After Onset of Ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP, mm Hg</td>
<td>118.7±21.2</td>
<td>121.2±19.4</td>
</tr>
<tr>
<td>PaO2, mm Hg</td>
<td>97.0±14.9</td>
<td>98.4±18.0</td>
</tr>
<tr>
<td>PacO2, mm Hg</td>
<td>30.4±2.7</td>
<td>31.6±4.8</td>
</tr>
<tr>
<td>Blood pH</td>
<td>7.394±0.04</td>
<td>7.369±0.08</td>
</tr>
<tr>
<td>Blood hematocrit</td>
<td>0.344±0.048</td>
<td>0.330±0.049</td>
</tr>
<tr>
<td>BT, °C</td>
<td>37.2±0.62</td>
<td>37.1±0.58</td>
</tr>
</tbody>
</table>

MABP indicates mean arterial blood pressure; BT, body (rectal) temperature. Values are means±SD (n=12) obtained before and 1 hour after MCA occlusion.

Statistical Analysis

The results are expressed as mean±SD. Changes in systemic parameters, ADC values, and water content were analyzed with one-way ANOVA and Scheffe’s F test. The relationships between the ADC and water content were assessed with linear regression analysis and the unpaired Student’s t test. Differences were considered significant at P≤.05.

Results

The physiological parameters were maintained within the normal ranges throughout the experimental period (Table 1). Perfusion imaging revealed perfusion deficits corresponding to the MCA territory in all 12 animals of groups 1 and 2. Mild (peak ΔR2* ratio >0.6 in the ROI), moderate (peak ΔR2* ratio between 0.6 and 0.2), and severe (peak ΔR2* ratio <0.2) perfusion deficits developed in 3, 5, and 4 animals, respectively (Table 2).

In the 3 animals showing mild perfusion deficits, no area of ADC reduction was detectable during the 3 hours after MCA occlusion. The ADC at the ectosylvian gyrus was 609±26.1×10^-6 mm²/s, which was not significantly different from the control group value (627±22.5×10^-6 mm²/s). In the animals showing moderate (n=5) and severe (n=4) perfusion deficits, areas of reduced ADC were detectable in the MCA territory as early as 15 minutes after ischemia onset, and these areas enlarged gradually thereafter. The ADC at 3 hours after ischemia onset at the ectosylvian gyrus declined significantly (P<.01) to 424±25.3 and 361±42.2×10^-6 mm²/s in the moderate and severe perfusion deficit groups, respectively. T2-weighted imaging showed very slight increases in 3 animals (Fig 1), whereas the other animal showed no detectable increases 3 hours after onset of occlusion.

In group 1 (n=6), 2 animals developed mild perfusion deficits. The tissue water contents of the gray matter at the ectosylvian gyrus of these animals remained within the normal range for this tissue (0.781 and 0.792 g water per gram tissue, respectively, versus mean control value 0.788±0.0067 g water per gram tissue), as did those of the white matter (0.667 and 0.664 g water per gram tissue, respectively, versus mean control value 0.663±0.0045 g water per gram tissue). The other 4 animals in group 1 developed moderate to severe perfusion deficits, and their gray and white matter tissue water contents at the ectosylvian gyrus increased significantly to 0.814±0.009 and 0.679±0.007 g water per gram tissue, respectively (both P<.01).

The correlations between the tissue water content and ADC were examined in the 4 animals that developed moderate to severe perfusion deficits. The tissue water content increases paralleled the ADC decreases in both the gray and white matter (Fig 2). The correlation lines for the gray and white matter were as follows.

Gray matter: y=−10105x+8533 (r=−.86)

White matter: y=−6174x+4611 (r=−.67)

where x is the water content (grams water per gram tissue), y is ADC (×10^-6 mm²/s), and r is the regression coefficient. The white matter values −6174 and 4611 were significantly different from the corresponding gray matter values (P<.05 and P<.01, respectively). Thus, both the slopes and intercepts of the correlation lines for the gray and white matter differed significantly.

No BBB opening to serum macromolecules, assessed by examining Evans blue extravasation, was detectable in the MCA areas of 5 of the group 2 animals, and the other animal showed very mild and localized Evans blue staining in the MCA area. Light microscopy revealed neuropilar microvacuolation and perivascular space enlargement in the ischemic gray matter. Many neurons showed mild cell body retraction with perineuronal space enlargement, and some neurons in the ischemic center showed cytoplasmic eosinophilia and nuclear pyknosis (Fig 3). Electron microscopy revealed hydric swelling of the astrocytic perikaryon, perivascular space, and perineuronal end-feet. Some neurons located in the ischemic center showed cell body retraction with increased electron density and nuclear pyknosis (dark neuron change).

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whereas many others appeared normal (Fig 4). No cell membrane disruption was observed in the neurons showing either of the above changes. In the ischemic white matter, edematous rarefaction of the myelinated fibers was observed light microscopically (Fig 3), and electron microscopy revealed marked cytoplasmic swelling of the oligodendroglia, as well as the astrocytes in the ischemic area. Furthermore, many myelinated fibers showed axonal swelling, and space formation between the myelin sheaths and axolemma was often observed (Fig 5).

Discussion

In this study we used an MCA occlusion/recirculation device designed for intramagnet use, which enable us to record MR images while keeping the animal under magnetism during the entire imaging period from before to after ischemia onset without artifacts caused by the use of metal. To minimize optic nerve injury during installation of the device, the eyeball was not removed for this procedure.

Various laboratory animals have been used to study ischemic edema. We used the cat because its white matter is well developed, making it suitable for regional ADC and water content measurement. Its cerebral architecture, which is similar to that of the human (ie, well-developed gyri and sulci with abundant semioval center and subcortical white matter) is another advantage, since edema fluid distribution has been shown to be influenced significantly by the white matter architecture.

In our cat model, the severity of ischemia varied considerably from very mild to severe. The incidences of moderate and severe ischemia were 5 of 12 and 4 of 12, respectively (Table 2). Similar variations in the severity of ischemia induced by MCA occlusion have been observed in the baboon and cat. The observed variation was suitable for analyzing the relationship between ADC change and ischemia severity.

We chose 3 hours as the time interval after onset of ischemia because this period is still within the therapeutic window and is the shortest interval in which treatment can be started in a clinical situation. In addition, our aim was to examine the ADC change during the cytotoxic phase of ischemic edema, which was within 6 hours after onset of focal cerebral ischemia in our previous study, as determined with a similar model.

In this study the ADCs of the control gray and white matter were $627 \pm 22.5$ and $562 \pm 31.1 \times 10^{-6}$ mm$^2$/s, respectively, in agreement with previous reports, although the white matter data tended to show wider variations than the gray matter data. We expressed our ADC data as absolute values, since quantitative ADC determination in a preparatory experiment showed high intra-animal and interanimal reproducibility. We obtained identical results by using the ADC ratio.

In a previous study we demonstrated that the white matter swelling during vasogenic edema showed anisotropy, which was dependent on the direction of the nerve fibers. Therefore, the ADC changes for the white matter due to ischemic edema may also show anisotropy. Our method of measuring the ADC involved irradiating diffusion sensitizing gradients in three different directions simultaneously, which avoids any influence of white matter ADC anisotropy during ischemic brain edema.

Using the same cat MCA occlusion model, we detected ischemic edema 6 hours after ischemia onset only at the

Figure 1. Representative MR images 3 hours after MCA occlusion. Shown are ADC map (a) and T2-weighted image (b) 10 mm anterior to the auditory meatus.

Figure 2. Correlation between water content and ADC of gray and white matter 3 hours after MCA occlusion.
ischemic center by CT scanning, whereas ADC mapping in this study showed ADC reduction as early as 15 minutes after ischemia onset. Thus, ADC mapping is a very sensitive method for detecting very early ischemic edema.

The extracellular space is considered the main determinant of the effective ADC. In ischemic tissue, the extracellular space becomes smaller as water shifts into the intracellular space because of increased intracellular osmolality and impairment of ion pumps in the cell membrane. Electron microscopic examination in this study showed cellular swelling in both the gray and white matter, which corresponded to the ADC decreases in both structures. Thus, ADC mapping is a powerful tool for detecting ischemic edema in the very early phase of cerebral

Figure 3. Light micrographs of ischemic gray (top left) and white (top right) matter and control gray (bottom left) and white (bottom right) matter (hematoxylin-eosin, magnification ×130).

Figure 4. Electron micrographs of ischemic gray matter. Swelling of astrocytic end-feet and an intact cortical neuron (left), a neuron showing “dark neuron change” (middle), and perivascular end-feet swelling (right) are shown. Bar=1 μm.
ischemia when the edema is still of the cellular subtype and therefore potentially curable.

However, care should be taken when the ADC value is used for quantitative evaluation of edema severity, since our data showed that both the water content corresponding to the ADC value and the rate of change for the gray and white matter differed significantly.

Cellular swelling (accumulation of water) in the gray matter takes place mainly in the astrocytes, whereas in the white matter we observed hydropic swelling of the oligodendroglial cell body, as well as the astrocytes. Intracellular water accumulation in the axon also occurred, and periaxonal space enlargement was seen in many myelinated fibers. These findings were in agreement with those reported recently by Pantoni et al. The observed different localizations of edema fluid in the ischemic gray and white matter probably account for the different slopes and intercepts of the ADC and water content correlation lines.

A close relationship between ADC reduction and tissue energy impairment has been reported. These studies showed that tissue ATP depletion corresponded to ADC reduction to 90±4% of the control level. However, whether there is a threshold ADC value for irreversible cell damage remains to be elucidated. Our study showed that a significant ADC decrease was seldom associated with cell membrane disruption or nuclear clamping, which are direct indicators of irreversible injury, in most of the ischemic areas observed, indicating that determination of an ADC threshold value for irreversible injury is difficult.

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References

Figure 5. Electron micrographs of ischemic white matter and control. Hydropic oligodendroglial and axonal swelling (top), some myelinated fibers showing myelin sheath splitting and periaxonal space enlargement (middle), and oligodendroglia in a control brain (bottom) are shown. Bar=1 μm.


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**Editorial Comment**

MRI is a noninvasive imaging approach. In relation to brain pathophysiology, the technique is potentially very powerful in that it can be used to assess development, progression, and regression of edema in vivo. MRI has been used previously to detect cerebral edema in experimental animals, and it is thought that the approach might be useful in defining areas of edema formation prior to onset of neurological signs.1

This study used MRI to examine early development of edema in an experimental model of focal ischemia. A key finding in the study relates to the observation that the MRI approach was able to detect very early changes in brain edema (as early as 15 minutes after onset of ischemia). Thus, this approach has the potential to be used to monitor early events related to stroke. The fact that repeated measurements can be made noninvasively with MRI raises the possibility that this approach would be useful experimentally to test the efficacy of early therapeutic interventions.

**Frank M. Faraci, PhD, Guest Editor**

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**Reference**

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