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 ($\times 10^{-6}$ mm$^2$/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

In 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. 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 pathophysologies 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. 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...
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 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 around it. The free ends of this thread were passed through two small holes in a small polyvinyl plate (2×4×1 mm), which was placed loosely on the MCA and then passed through a polypropylene tube (3-mm ID, 20 mm long) that was anchored to the orbit with dental cement after the craniectomy opening had been sealed with an absorbable gelatin magnet.

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

Magnetic Resonance Imaging

MRI was performed with the use of a 4.7-T experimental imager/spectrometer system (Unity INOVA) with a 330-mm horizontal bore magnet equipped with shielded gradients (maximal strength, 65 mT/m) and a 160-mm-ID quadrature detection coil. The animal was kept under 1.5% isoflurane anesthesia and immobilized with the use of a workstation-based image analysis system (Spark 10, SUN). ADC maps were generated from two-point analysis on a pixel basis with the use of the following standard equation: \[ \text{ADC} = \ln \left( \frac{S_0}{S_t} \right) / (b_1 - b_0), \] where \(S_0\) and \(S_t\) are the signals of the two DWIs representing the average of the three values in three orthogonal planes, i.e., a trace of the diffusion tensor.

From the T2 image before MCA occlusion, ROIs in the gray and white matter of the cingulate, middle suprasylvian, middle ectosylvian, and posterior sylvian gyri and in the semioval centers on both sides were drawn, and the ADC value corresponding to each of these ROIs at each time interval was determined. The ratio of the ADC value on the occluded side to that of the corresponding ROI in the contralateral hemisphere was also calculated.

Furthermore, perfusion imaging was performed according to the principles and techniques reported previously. 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 gadopentetate 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 gyri on the coronal image were measured, and these data were transformed into plots of \(\Delta R^*_s\) versus time, according to the following formula:

\[ \Delta R^*_s = -\ln[\text{S}(t)/S(0)]/TE \]

where \(TE\) is the echo time, \(S(t)\) is the image intensity at time \(t\), \(S(0)\) is the precontrast baseline signal intensity, and \(\Delta R^*_s\) is the change in effective transverse relaxation rate. The ratio of the peak ischemic to control tissue \(\Delta R^*_s\) 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

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 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.
MABP, mm Hg 118.7

D between 0.6 and 0.2, and severe (peak to the MCA territory in all 12 animals of groups 1 and 2. Mild Perfusion imaging revealed perfusion deficits corresponding normal ranges throughout the experimental period (Table 1).

The physiological parameters were maintained within the analysis and the unpaired Student’s F test. The relationships between parameters, ADC values, and water content were assessed with linear regression one-way ANOVA and Scheffe’s F test. The relationships 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 ($\times 10^{-6}$ mm$^2$/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.

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 ($\times 10^{-6}$ mm$^2$/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 gray matter. Many neurons showed mild cell body retraction with perivascular space enlargement in the ischemic gray matter. Many neurons showed mild cell body retraction with perivascular space enlargement, and some neurons in the ischemic center showed cytoplasmic eosinophilia and nuclear pyknosis (Fig 3). Electron microscopy revealed hydropic swelling of the astrocytic perikaryon, perivascular space, and perivascular end-feet. Some neurons located in the ischemic center showed cell body retraction with increased electron density and nuclear pyknosis (dark neuron change).

### Table 2. Variations in Ischemic Severity Classified According to Peak $\Delta R^*_2$ Ratio and ADC Changes in Gray Matter Perfused by the MCA After Occlusion

<table>
<thead>
<tr>
<th>Ischemia</th>
<th>Control</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Peak $\Delta R^*_2$ ratio</td>
<td>1.02±0.078</td>
<td>0.746±0.15</td>
<td>0.421±0.15</td>
<td>0.088±0.009</td>
</tr>
<tr>
<td>ADC, $\times 10^{-6}$ mm$^2$/s</td>
<td>627±22.5</td>
<td>609±26.1</td>
<td>424±25.3*</td>
<td>360.5±42.2*</td>
</tr>
</tbody>
</table>

Mild, moderate, and severe ischemia correspond to peak $\Delta R^*_2$ ratios >0.6, between 0.6 and 0.2, and <0.2, respectively. The peak $\Delta R^*_2$ ratio and ADC values were obtained at the ROIs in the gray matter of the left ectosylvian gyrus 30 minutes (peak $\Delta R^*_2$ ratio) and 3 hours (ADC) after onset of ischemia.

*Significantly different from control value ($P<.01$).
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 semi-oval center and subcortical white matter) is another advantage, since edema fluid distribution has been shown to be influenced significantly by the white matter architecture.8

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 baboon9 and cat.10 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,11,12 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 variations3,13,14 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.15 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
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.

Acknowledgments

This study was supported in part by a project grant from Tokyo Metropolitan Institute of Gerontology. We wish to thank Dr Shizuko Ichinose, Dr Shu Endo, and Yoshie Furusawa for their excellent assistance with this study. We are grateful for the helpful contributions and support of Profs R. Okeda, A. Tamura, and K. Hirakawa during this project.

References

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
Department Internal Medicine
Cardiovascular Division
University of Iowa College of Medicine
Iowa City, Iowa

Reference

Different Apparent Diffusion Coefficient: Water Content Correlations of Gray and White Matter During Early Ischemia
Toshihiko Kuroiwa, Tsukasa Nagaoka, Masato Ueki, Ichiro Yamada, Naoyuki Miyasaka and Hideaki Akimoto

*Stroke*. 1998;29:859-865
doi: 10.1161/01.STR.29.4.859

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
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:
http://stroke.ahajournals.org/content/29/4/859

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/