Experimental ischemic brain edema: morphological and magnetic resonance imaging findings

TOSHIHIKO KUROIWA, M.D.,¹ NAOYUKI MIYASAKA, M.D.,² ZHAO FENGYO, M.D.,³ ICHIRO YAMADA, M.D.,⁴ MAKOTO NAKANE, M.D.,⁵ TSUKASA NAGAOKA, M.D.,⁶ AKIRA TAMURA, M.D.,⁵ AND KIKUO OHNO, M.D.⁶

¹Department of Pathophysiology, Medical Research Institute; Departments of ²Obstetrics and Gynecology, ³Neurology, ⁴Radiology, and ⁵Neurosurgery, Tokyo Medical and Dental University; and ⁶Department of Neurosurgery, Teikyo University, Tokyo, Japan

✓Magnetic resonance (MR) imaging is a powerful tool for detecting and characterizing ischemic edema, a serious complication of ischemic cerebrovascular disorders. In this article the authors investigate the relationships between MR imaging findings and structural/ultrastructural changes in ischemic brain edema by using various animal models of experimental cerebral ischemia. The authors observed the following: 1) Ischemic edema was detectable by diffusion weighted MR imaging as early as 15 minutes after the onset of vascular occlusion. A decrease in the apparent diffusion coefficient (ADC) corresponded to the early cellular/cytotoxic type of brain edema and the decrease was proportionate to the degree of intracellular water accumulation. 2) Postischemic transient normalization of the ADC after the initial decrease did not signify histological recovery but rather slowly progressing infarction. 3) Histological degradation of postischemic tissue correlated with the decrease in tissue elasticity and the magnetization transfer ratio. 4) Transient cytotoxic edema localized in the substantia nigra preceding neuronal death was detectable on MR images after ipsilateral striatal infarction. Thus, MR imaging is a powerful tool for detecting and characterizing brain edema associated with ischemic stroke.

KEY WORDS • apparent diffusion coefficient • ischemic brain edema • magnetic resonance imaging • magnetization transfer ratio • tissue elasticity • ultrastructural change

ISCHEMIC EDEMA appears almost simultaneously with the onset of ischemia.^{4,6} The condition is known to be heterogeneous in nature, which is cellular at the onset and is exacerbated by the superimposition of a vasogenic component. Magnetic resonance imaging is a powerful tool used to detect and characterize ischemic edema.^{2,6} In this article we present a summary of our previous studies of ischemic edema in various animal models of cerebral ischemia based on MR imaging and morphological findings.^{6,8,12,13,24,25} Early and accurate diagnosis of ischemic edema is essential for the prevention of a deleterious outcome. Diffusion weighted MR imaging enables detection of ischemic edema during the acute phase when most of the damage caused by ischemia is still reversible and there-

fore within the therapeutic window. However, it has also been shown that DW images show transient normalization after short-duration ischemia that is followed by secondary deterioration.¹⁵ It has not been clarified whether transient normalization seen on DW MR imaging indicates a histological recovery. We examined the ultrastructural localization of water in tissue at the acute phase of ischemic edema showing an initial decrease of water diffusion by ADC mapping (Part I).⁶ Given that transient normalization of DW MR imaging is a clinically important phenomenon for selecting appropriate treatment, we then examined the relationship between the temporal changes of ADC and structural/ultrastructural tissue changes focusing on the postischemic transient normalization (Part II).^{12,13} Ischemic edema causes brain herniation, and herniation causes tissue deformation and secondary ischemia/hemorrhage. These processes should be significantly influenced by the changes in the biomechanical property of the ischemic brain tissue. Biomechanical data

Abbreviations used in this paper: ADC = apparent diffusion coefficient; CCA = common carotid artery; DW = diffusion weighted; GABA = γ -aminobutyric acid; MCA = middle cerebral artery; MR = magnetic resonance; MTR = magnetization transfer ratio; ROI = region of interest; SN = substantia nigra.

of diseased brain tissue should therefore be important to understand the mechanism of postischemic brain tissue injury. However, available data at present are scarce. The MR imaging MTR is a parameter of water molecules bound to large-molecular-weight structures. The MTR has been shown to be sensitive to structural changes of the central nervous system tissue (for example, demyelination).¹⁰ We examined the time course of regional tissue elasticity by applying an indentation method on *ex vivo* brain tissue and correlated it with tissue water content and MR imaging findings (Part III).⁸ Neuronal death develops slowly in the SN after focal cerebral ischemia involving the ipsilateral striatum.²² The SN has synaptic terminals of GABAergic neurons from the ipsilateral striatum, and the absence of GABAergic inhibitory signal due to infarction probably induces excessive neuronal firing, astrocytic swelling, and subsequent neuronal death in the SN.²⁰ On T2-weighted MR images a transient high signal intensity is seen in the SN after infarction in the striatum in both humans and rodents,^{17,18} indicating transient localized edema of the SN. We examined the time course and relationships between MR imaging findings and ultrastructural changes of the SN after striatal infarction (Part IV).^{24,25}

Materials and Methods

All animal experiments were performed according to protocols approved by the Committee on Animal Research of Tokyo Medical and Dental University. Magnetic resonance imaging was performed using a 4.7-tesla experimental imaging/spectrometer system (Unity INOVA) with a 330-mm horizontal bore magnet equipped with shielded gradients (maximum strength 65 milliteslas/m) and a 160-mm (inner diameter) quadrature detection coil. Perfusion imaging was performed according to the principles and techniques reported previously. All data analyses were performed using a SunSparc 10 workstation (Sun Microsystems) and image analyzing software (XDS, Davis Bioengineering). The detail of the MR imaging procedure was described in our previous publications.^{6,8,12,13,24,25}

Part I: The ADC and Postischemic Ultrastructure

Fifteen adult cats (weight 3.5–4.5 kg) were anesthetized with isoflurane, artificially ventilated, and kept normothermic during the experiment. An MCA occlusion/recirculation device⁷ was implanted in each animal through a transorbital approach for the intramagnet occlusion and recirculation of the MCA. The animal was placed in the experimental MR imaging unit. We repeatedly performed T2-weighted imaging, DW imaging, and perfusion imaging for 180 minutes after MCA occlusion. Brain edema was assessed using tissue gravimetry. Each animal was killed with an overdose of pentobarbital, a coronal brain slice corresponding to the level of ADC map was cut, and tissue samples from the sites corresponding to ROIs in the gray and white matter were excised for tissue gravimetry. Tissue water content determined by the gravimetry was compared with the ADC value for the same ROI. Blood–brain barrier permeability to serum proteins was assessed by examining leakage of Evans blue dye injected intravenously shortly after MCA occlusion. To examine

FIG. 1. Perfusion image (*left*) and ADC map (*right*) obtained 3 hours after MCA occlusion.

the structural and ultrastructural changes corresponding to the ADC changes, the animal was perfused transcardially with a buffered fixative shortly after the final MR imaging procedure. The brain was removed and sampled for electron microscopic examination. The mirror surface coronal section was then prepared and stained with H & E and cresyl violet for light microscopic examination.

Part II: Time Course of ADC and Histological Outcome

In this experiment, 15 adult male Sprague–Dawley rats were used. The animals were anesthetized with isoflurane, kept in normothermic conditions, and subjected to left CCA ligation. The animals underwent unilateral CCA ligation alone, sham operation and hypoxia, or both unilateral CCA ligation and hypoxia for 15, 30, or 60 minutes. Magnetic resonance imaging was performed before hypoxic/ischemic insult, and 60 and 48 hours after the hypoxic/ischemic insult. The ROIs for the ADC measurements were selected in the ipsilateral parietal cortex of a coronal section. After the final MR imaging procedure, the brain was perfusion-fixed with the animal in a state of deep anesthesia and it was then removed. A coronal slice corresponding to the ADC map was cut and prepared for light microscopy examination or electron microscopy examination.

Part III: Tissue Elasticity After Ischemia

For biomechanical examination, 20 adult Mongolian gerbils (weight 60–75 g) were used. The animal was subjected twice to 10 minutes of occlusion of the left CCA after induction of isoflurane anesthesia at 5-hour intervals. The animal that exhibited stroke symptoms¹⁹ was selected for further analysis. Magnetic resonance imaging was performed repeatedly after ischemia for T2 signal and ADC mapping. The animal was killed shortly after the final MR imaging procedure by an overdose of pentobarbital. The brain was removed, cut 3 mm thick coronally at the level of the chiasm, and kept in a container with silicon oil at 37°C. *Ex vivo* elastic response of the brain tissue was measured by applying an indentation method using a pressure-displacement transducer.^{1,23} After elasticity measurement, the specific gravity of the tissue corresponding to the measurement was ascertained, and the tissue water content was calculated. The coronal slice with the mirror surface was immersion fixed in 4% buffered formalin and

FIG. 2. Light micrographs of control gray matter (A), ischemic gray matter (B), control white matter (C), and ischemic white matter (D). Electron micrographs of ischemic gray matter showing swollen astrocytic endfeet (E), dark neurons (F), and perivascular endfeet swelling (G). Electron micrographs of control white matter (H) and ischemic white matter showing hydropic swelling of oligodendrocyte (I). H & E, original magnification $\times 260$ (A–D); bar = 1 μm (E–I).

prepared for light microscopic examination with H & E, Kluever Barrera, and Bodian staining.

Part IV: MR Imaging Detection of Remote Postischemic Edema

Twenty adult male Sprague–Dawley rats were anesthetized with halothane, and the left MCA was permanently

occluded via a retroorbital approach.¹⁹ Magnetic resonance imaging was performed at 1, 4, 7, and 12 days after MCA occlusion or the sham procedure. Shortly after the MR imaging procedure, the rats underwent transcardiac perfusion, and the brains were cut coronally into sequential slices corresponding to the MR images. Both SNs were excised from the slice at the level of the medial terminal nucleus of the accessory optic tract. One-micrometer-thick sections were stained with 1% toluidine blue and

FIG. 3. The ADC maps (A, E, I, and M), light micrographs (B, F, J, and N), and electron micrographs (C, D, G, H, K, L, O, and P) obtained in a control rat (A–D) and in experimental rats during primary ADC reduction (E–H), transient ADC recovery (I–L), and secondary ADC reduction (M–P) phases. Note microvacuolation of the neuropil with pyknotic neurons (*asterisk*) at the transient ADC recovery phase in panel J. Hydropic swelling of astrocytic endfeet (*number signs*) was observed using an electron microscope at this phase in Panels K and L. Original magnification $\times 260$ (B, F, J, and N); bar = $1\ \mu\text{m}$ (C, D, G, H, K, L, O, and P).

examined under light microscopy. Ultrathin sections were prepared for electron microscopic examination.

Statistical Analysis

All data are expressed as the mean $\pm$ standard deviation. Data were analyzed by using one-way analysis of variance and the Scheffé F-test. Differences were considered significant at a probability level less than 0.05.

Results

Part I: The ADC and Postischemic Ultrastructure

The physiological parameters were maintained within the normal ranges throughout the experimental period. Perfusion imaging revealed perfusion deficits in the MCA territory (Fig. 1 *left*). The reduction in ADC was detectable in the areas showing the perfusion deficit 15 minutes

after occlusion and thereafter (Fig. 1 *right*). The water content was measured by tissue gravimetry and increased from 78.8 ± 0.67 to $81.4 \pm 0.9\%$ in the gray matter and 66.3 ± 0.45 to $67.9 \pm 0.7\%$ in the white matter. The increases of water content correlated linearly with the ADC decreases in both the gray and white matter. Correlation between the percentage of water content and the ADC value in the gray matter and white matter was as follows: $y = -101.05x + 8533$ ($r = 0.86$) and $y = -61.74x + 4611$ ($r = 0.67$), respectively, where x is the percentage of water content and y is the ADC ($\times 10^{-6}\ \text{mm}^2/\text{second}$). Thus, both percentage of water content corresponding to an ADC value and the rate of ADC change of the gray and white matter differed significantly, as shown in the equations. No blood–brain barrier opening to serum proteins was detectable in the MCA areas at 3 hours after onset of ischemia. Based on light microscopy findings, neuropilar microvacuolation, perivascular space enlargement, cytoplasmic eosinophilia, and nuclear pyknosis of neurons

Fig. 4. Graphs showing the time course of percentage of tissue elasticity (*upper*) and percentage of MTR (*lower*). The *solid line* denotes the experimental study and the *dotted line*, control. * $p < 0.05$.

were found in the ischemic gray matter (Fig. 2A and B). Edematous rarefaction of the myelinated fibers with a perinuclear halo of oligodendrocytes was observed in the ischemic white matter (Fig. 2C and D). Electron microscopy revealed hydropic astrocytic swelling in both structures. In the gray matter, some neurons showed cell body retraction with increased electron density and nuclear pyknosis, whereas many others appeared normal. However, no cell membrane disruption was observed in either type of neuron (Fig. 2E–G). In the white matter, oligodendroglial and myelinated axonal swelling and periaxonal space enlargement were observed (Fig. 2I).

Part II: Time Course of ADC and Histological Outcome

The animals subjected to the hypoxic/ischemic insult showed three different patterns of ADC change depending on the duration of the insult. Transient ADC reduction—reduction during the insult and recovery after resuscitation—was found in the animals subjected to the 15-minute hypoxic/ischemic insult. Biphasic ADC reduction—transient ADC recovery after the insult—was found in the animals subjected to 15-, 30-, or 60-minute hypoxic/ischemic insult. Persistent ADC reduction pattern—no recovery after the insult—was found in the animals subjected to 60-minute hypoxic/ischemic insult. The transient ADC re-

Fig. 5. Light micrographs of control gray matter (A) and gray matter at 5 hours (B), 4 days (C), and 7 days (D) postischemia. Bodian staining, original magnification $\times 260$.

duction pattern was associated with selective neuronal death. The biphasic and persistent ADC reduction patterns were associated with cerebral infarction.

In the biphasic ADC reduction animals, electron microscopy revealed dendritic swelling of neurons and mild swelling of the perivascular astrocytic endfeet during the primary ADC reduction phase (Fig. 3G and H). During the transient ADC recovery phase, dark neurons and pronounced swelling of the perivascular astrocytic endfeet were observed in the posthypoxic/ischemic tissue (Fig. 3J–L). During the secondary ADC reduction phase, severely retracted dark neurons and extensive swelling of the astrocytic endfeet were observed (Fig. 3N–P).

Part III: Tissue Elasticity After Ischemia

Ischemic edema in this model was detectable in the left forebrain 5 hours postischemia and thereafter. Infarction developed slowly during 7 days postischemia. Elasticity measurement showed the following three stages of biomechanical change after ischemic insult (Fig. 4). Stage I: In the early postischemia period (0–5 hours), tissue elasticity remained within the normal range, despite a mild increase in the tissue water content. Mild vacuolation of neuropil with intact neural fibers was observed in the postischemic tissue (Fig. 5). The ADC significantly decreased, T2 signal mildly increased, and MTR remained within normal range (Figs. 4 and 6). Stage II: At this stage (5 hours–4 days), tissue elasticity mildly decreased in parallel to the increase of tissue water content. Diffuse ischemic neuronal change with neuropil microvacuolation was evident. The ADC gradually returned to the baseline, whereas the T2 signal further increased and MTR mildly decreased. Stage III: In the late postischemic brain (4 days–4 weeks), tissue elasticity decreased to the lowest level. Numerous infiltrations of macrophages with loss of normal tissue structure were found in the postischemic tissue. The ADC and T2 signal remarkably increased, whereas the MTR decreased significantly. Thus, the time course

FIG. 6. The ADC maps (*upper*) and MTR maps (*lower*) obtained in a control animal (A and D) and an experimental animal 5 hours (B and E) and 7 days (C and F) postischemia.

of tissue elasticity is similar to that of MTR (Figs. 4 and 6).

Part IV: MR Imaging Detection of Remote Postischemic Edema

Electron microscopy examination on Day 1 revealed dotted chromatin clumps in the nuclei of some neurons and mild swelling of the perivascular endfeet of astrocytes in the ipsilateral SN. The T2-weighted images revealed high signal intensity indicative of infarction in the ipsilateral caudate nucleus, putamen, and cortex but not the SN. Diffusion weighted images similarly failed to reveal any changes in the SN [Fig. 7A–C]. On Day 2, a few neurons

of the ipsilateral SN revealed apoptotic body-like condensation of the nuclear chromatin indicating an apoptosis process in some dying neurons in the SN. On Day 4, nearly half of the neurons in the SN showed hydropic swelling, and the neuropil became strongly roughed and loose (Fig. 8A, C, and D). Cytoplasmic microvacuoles apparently originating from rough endoplasmic reticulum, mitochondria, and Golgi apparatus were also frequently observed. On Day 4 the DW images revealed high signal intensity in the ipsilateral SN, in which the ADC transiently and significantly decreased and the T2 signal value significantly increased (Fig. 7D–F). The severity of cellular swelling paralleled the change in the ADC. Reactive astrocytes and dark neurons most frequently appeared on Days 7 and 12. The T2 signal and ADC returned to and remained at control levels on Days 7 and 12 (Fig. 7G–I).

Discussion

In Part I, we induced MCA occlusion in cats to analyze the ADC changes in the gray and white matter. The white matter is well developed in cats and the architecture is similar to that in humans; thus, this model is suitable for the analysis of brain edema in the white matter.⁹ We found that a decrease in the ADC, signifying cellular edema, is detectable as early as 15 minutes after onset of ischemia both in the gray and white matter. Electron microscopy revealed intracellular accumulation of edema fluid mainly in the astrocytes in the gray matter. However, water accumulation was remarkable also in the oligodendrocytes and axons in the white matter. The different distribution of

Fig. 7. Diffusion weighted images (A, D, and G), ADC maps (B, E, and H), and T2-weighted images (C, F, and I) obtained in an experimental rat before (A–C), 4 days after (D–F), and 7 days after (G–I) MCA occlusion. The asterisk indicates the ipsilateral SN.

Fig. 8. Light micrographs of the SN on Days 4 (A) and 7 (B) after MCA occlusion. Electron micrographs of an SN neuron (C) and perivascular endfeet of astrocyte (D) on Day 4, both showing hydropic swelling. Bar = 1 μ m

water observed ultrastructurally probably resulted in the different water content corresponding to the ADC value and rate of change for the gray and white matter. This difference should be taken into account when the ADC value is used for quantitative evaluation of edema severity. Thus, ADC mapping is a powerful tool for detecting ischemic edema in the very early phase when the edema is still of the cellular subtype and potentially reversible.

Sequential MR imaging in rats subjected to hypoxic/ischemic insult revealed that there are three different time course patterns in ADC changes, depending on the duration of the hypoxic/ischemic insult: transient, biphasic, and persistent ADC reduction patterns. Histologically, the transient pattern correlated with selective neuronal death. Biphasic and persistent patterns were associated with cerebral infarction. These findings indicate that sequential DW MR imaging is a potentially powerful tool in the evaluation of hypoxic/ischemic brain injury. It has been shown that early reperfusion both in experimental and human brain ischemia can reverse acute ADC reductions and that DW MR imaging can delineate potentially reversible ischemic brain changes.^{5,11} However, the available data have shown that transient ADC normalization after cerebral hypoxic/ischemic insult does not necessarily mean histological recovery. Thus, transient ADC recovery probably has limited use for assessing postischemic tissue recovery.

To measure biomechanical properties of edematous brain tissue, we applied an indentation method on the *ex vivo* brain tissue.^{1,23} With this procedure, we could measure regional biomechanical properties of small-sized brain tissue. Changes in elasticity of the postischemic edematous brain tissue could be separated into three stages: Stage I (0–5 hours postischemia), Stage II (5 hours–4 days postischemia), and Stage III (4 days–4 weeks postischemia). In Stage I, elasticity did not change despite early ischemic edema. Cellular swelling was the main histological finding. In Stage II, an increase of water content par-

alleled the decrease in tissue elasticity. Ischemic tissue swelling without apparent tissue necrosis developed in this stage. In Stage III, elasticity significantly decreased, and tissue necrosis became evident. The MTR mapping showed a time course similar to that of elasticity change. The MTR reflects the density of water molecules bound to large molecular structures. The decrease probably reflects the decrease in the amount of water molecules bound to the structures. Magnetic resonance imaging elastography has been developed to measure directly the biomechanical property of tissue *in vivo*.¹⁴ Future application of this technology to the brain would be important for understanding the biomechanical changes of edematous brain tissue.

Focal cerebral infarction induces neuronal death in remote brain regions that are innervated by neurons located at the primary lesion site. In humans, it has been reported that infarction involving the caudate nucleus and putamen causes remote postischemic neuronal death in the SN.³ Magnetic resonance imaging performed after caudate infarction showed transient diffusion abnormality in the SN.¹⁸ These results indicate that MR imaging can detect remote edematous changes in the SN associated with cerebral ischemia in humans. We observed this phenomenon in rats subjected to permanent occlusion of the MCA. The SN is not ischemic after MCA occlusion in rats. In fact, the regional cerebral blood flow and glucose utilization by the ipsilateral SN are increased.²¹ Therefore, the changes in the SN are not due to ischemia. The level of the inhibitory neurotransmitter GABA is reported to be decreased in the ipsilateral SN after MCA occlusion in rats, which is likely to trigger spontaneous firing of the SN neurons and subsequent neuronal death.^{18,20} The MR imaging data revealed a transient decrease in the ADC value, whereas the T2 signal increased in the ipsilateral SN on Day 4. Both values returned to normal levels on Day 7 and 12. These data are indicative of delayed brain edema localized in the ipsilateral SN prior to the neuronal death. Thus, we could detect localized edema proceeding to neuronal death of SN after MCA occlusion. Electron microscopy revealed that the neuronal death in the SN after MCA occlusion was due to both apoptosis and necrosis, although necrosis seems to be the dominant mechanism of the neuronal death.

Diffusion weighted MR imaging is a powerful tool for the detection and characterization of ischemic edema. Early and accurate diagnosis is possible by using this modality. Understanding the advantage and limitation of this procedure is important for its effective usage.

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Address reprint requests to: Toshihiko Kuroiwa, M.D., Department of Pathophysiology, Medical Research Institute, Tokyo Medical and Dental University, Bunkyo-ku 1–5–45, Tokyo 113–8510, Japan. email: tkuroiwa.nsu@umin.ac.jp.