Novel Brain Ischemic Change on MRI
Delayed Ischemic Hyperintensity on T1-Weighted Images and Selective Neuronal Death in the Caudoputamen of Rats After Brief Focal Ischemia
Masayuki Fujioka, MD; Toshiaki Taoka, MD; Yoshiyuki Matsuo, PhD; Ken-Ichiro Hiramatsu, MD; Toshisuke Sakaki, MD

Background and Purpose—Specific change of persistent hyperintensity/hypointensity on T1-weighted (T1W) and T2-weighted (T2W) MRI, respectively, has been reported to develop in the human basal ganglia after brief hemispheric ischemia. We investigated whether this ischemic change observed in humans could be reproduced experimentally in rats after brief middle cerebral artery (MCA) occlusion (MCAO), and if so, what the neuroradiological change represented histologically.

Methods—The origin of the right MCA of male Wistar rats (n = 25) was occluded for 15 minutes by inserting a silicon-coated nylon thread from the external carotid artery into the internal carotid artery. After 15 minutes’ MCAO, coronal MR images (T1W, T2W, and T1W with fat saturation pulse) were obtained once at 3-day reperfusion (n = 5) and twice at 3- and 7-day reperfusion (n = 20). Brain specimens were examined histologically immediately after the last MRI study in all rats.

Results—Neither T1W nor T2W MRI showed marked signal changes 3 days after reperfusion following 15-minute MCAO. However, the ischemic change of hyperintensity and hypointensity on T1W and T2W MRI, respectively, appeared in the striatum following 7-day reperfusion after 15-minute MCAO (n = 19/20). Histological examination revealed that the specific lesion in the rat striatum on MRI corresponded to selective neuronal death and proliferation of reactive astrocytes and microglia without infarct, hemorrhage, lipid accumulation, or calcification.

Conclusions—Brief MCAO with reperfusion induces the delayed ischemic changes of hyperintensity and hypointensity on T1W and T2W MRI, respectively, in the rat striatum with high reproducibility. This specific ischemic change on MRI histologically corresponded to selective neuronal death and gliosis with preservation of the macroscopic structure of the brain. A similar MRI pattern reported in patients who have sustained brief ischemia may represent similar histology. We speculate that the ischemic change reflects some biochemical changes affecting the magnetic field as the brain tissue undergoes subtle structural changes. (Stroke. 1999;30:1043-1046.)

Key Words: cerebral ischemia, transient ■ magnetic resonance imaging ■ middle cerebral artery ■ neuronal death ■ rats

Brief middle cerebral artery (MCA) occlusion (MCAO) induces selective neuronal death and various glial responses (incomplete infarction) without pan necrosis of the tissue and subsequent cavitation.4 This subtle morphological change caused by mild ischemia has been strongly believed to appear normal on conventional CT scans and MR images.1-3 However, we suggested in our clinical study that a delayed change on MRI represented incomplete ischemic injury, including selective neuronal death and gliosis without infarct or hemorrhage.4 This ischemic change exhibited persistent hyperintensity and hypointensity on repeated T1-weighted (T1W) and T2-weighted (T2W) MRI, respectively, with the hyperintensity on T1W imaging gradually subsiding with time. Brief cerebral hemispheric ischemia leading to spectacular shrinking deficit produced the specific ischemic change in the basal ganglia and cerebral cortex in humans on MRI but not CT scans.4

The objective of the present study was to investigate whether this ischemic change of hyperintensity and hypointensity on T1W and T2W MRI observed in the striatum of humans could be reproduced experimentally in rats after brief MCAO, and if so, what the specific neuroradiological change represented histologically.

Materials and Methods

Animals
Male Wistar rats (Slc Experimental Animal Co Ltd, Shizuoka, Japan) weighing 270 to 320 g were used throughout this study (n = 33). These animals were housed in groups of 2 to a room, with controlled temperature (24±1°C) and humidity (55±5%) under a 12-hour light/dark cycle (lights on from 8:00 AM to 8 PM). They were allowed...
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free access to food and water. All experiments were in accordance with our institutional guidelines.

Study Design
Animals were divided into 3 experimental groups. The first group was subjected to right transient MCAO for 15 minutes followed by reperfusion lasting for 3 to 7 days (n=25). The second group was subjected to right transient MCAO for 60 minutes followed by reperfusion lasting for 7 days (n=5). The third group had the right MCA occluded for approximately 10 seconds followed by reperfusion for 7 days (n=3). The first group underwent MRI twice at 3- and 7-day reperfusion (n=20) and once at 3-day reperfusion (n=5). The second group underwent MRI twice at 3- and 7-day survival (n=5), and the third group underwent MRI twice at 3- and 7-day reperfusion (n=3). Brain specimens were examined histologically immediately after the last MRI study in all rats.

Surgical Procedure
The right MCA of the rat was occluded, as previously described by a coauthor of this study. Briefly, rats were anesthetized with a gas mixture of 98% air and 2% halothane. After a median incision of the neck skin was made, the right external carotid artery was carefully dissected and an 18-mm length of 4-0 nylon thread, precoated with silicon, was inserted from the lumen of the external carotid artery to that of the right internal carotid artery to occlude the origin of the right MCA. Body temperature was maintained at 37°C with a heating pad. The surgery was performed within 8 minutes without bleeding. After the surgery, anesthesia was discontinued and the rats were allowed free access to food and water until removal of the thread. Neurological deficits characterized by left forepaw paresis and Horner’s syndrome were strictly used as criteria for 15-minute and 60-minute MCAO as significant ischemic insults. Rats with convulsions and/or disturbances of consciousness mainly due to artificial hypothermia were then excluded from this study. The rats with mild to moderate forepaw paresis were also excluded. After 15 minutes (n=25), 60 minutes (n=5), and 10 seconds (n=3) of MCAO, the thread was removed to allow complete reperfusion of the ischemic area via the right common carotid artery. In the rats subjected to 15-minute MCAO, the left forepaw paralysis began to disappear within 10 minutes after reperfusion.

MRI Study
In MRI studies, rats were anesthetized with chloral hydrate (400 mg/kg IP) and fixed to a Taoka rat cradle. They maintained respiration without assistance. A 3-inch-diameter circular receive-only surface coil was placed under the rat head, with the center of the coil located at the midpoint of the midline between the ear-ear and eye-eye lines. Body temperature was kept at 37°C with a heating pad. The temperature of the MR imaging room was controlled at around 27°C.

MRI Parameters
MR imaging was performed with a clinical imaging system at 1.5T (General Electric Signa Advantage MR system). Coronal T1W, T2W, and T1-W with fat saturation (FS) pulse sequences were obtained using a spin-echo technique (TR=500 ms and TE=20 ms for the T1W images; TR=3000 ms and TE=100 ms for the T2W images; and TR=500 ms and TE=20 ms with FS pulse for the FS images) between the pole of the frontal lobe and the most caudal portion of the cerebellum. Other imaging parameters included 2-mm slice thickness with 1-mm gap junction, matrix size 256×128, and 8×4 cm field of view.

Histological Examination
For the histopathological studies, all rats were perfused transcardially with 100 mL of physiological saline followed by 10% formalin (25°C at a pressure of 100 mm Hg) immediately after the last MRI studies. Brains were removed carefully, postfixed in formalin, and embedded in paraffin. Then 5-μm-thick brain sections were processed for hematoxylin and cosin (H&E) staining and immunohis-
tochemical demonstration of glial fibrillary acidic protein (GFAP), and thereafter examined with a light microscope. Histopathological evaluation was made with reference to the definitions reported by Garcia et al. Neuronal death was identified when at least 1 of the following alterations was found by light microscopy: pyknosis, karyorrhexis, and karyolysis, as well as cytoplasmic cosinophilia or loss of affinity for hematoxylin. Selective neuronal death was characterized by ischemic injury limited to specific populations of brain neurons, with sparing of glial cells and microvessels. Infarct (pannecrosis) meant histological changes, including the loss of affinity for hematoxylin in all types of cells, including neurons, glial cells, and blood vessels. Stains for iron, manganese, calcium, and lipid were not performed.

Results

Controls
The sham-operated rats had no detectable ischemic brain lesions in either the MR imaging or histological studies (Figure 1D). In rats after 60-minute MCAO (Figure 1A, 1B, and 1C), serial MR images demonstrated ischemic lesions of hypointensity and hyperintensity on T1W and T2W images, respectively, in the caudoputamen and cerebral cortex of the MCA territory (A, B), which is histologically consistent with infarction (C: H&E, magnification ×132). Note the appearance of early cavitation and monocyte infiltration. Histological examination shows no abnormal findings in the MCA region in sham-operated rats (D: H&E, magnification ×132).

15-Minute MCAO Models
MRI obtained 3 days after the onset of reperfusion showed no marked signal changes in the ipsilateral hemisphere in 21 rats (n=21/25, 84%), slight hypointensity on T2W images in the striatum in 3 rats (n=3/25, 12%), and slight hyperintensity on T2W images in the dorsolateral caudoputamen in 1 rat (n=1/25, 4%) (Table, Figure 2). Following 7-day reperfusion after 15-minute MCAO, however, the specific ischemic change of hyperintensity and hypointensity on T1W and T2W MRI, respectively, appeared in the striatum in 19 rats (n=19/
Serial Change in the Caudoputamen of Rats After 15-Minute MCAO

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<th>Day 3</th>
<th>Day 7</th>
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<tr>
<td>T1W/T2W imaging</td>
<td>Iso/iso (n=21/25)</td>
<td>High/low (n=19/20)</td>
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<td></td>
<td>Iso/sl-low (n=3/25)</td>
<td>Iso/iso (n=1/20)</td>
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<td>Iso/sl-high (n=1/25)</td>
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<td>Histology</td>
<td>SND, gliosis nervous tissue (n=24/25)</td>
<td>SND, gliosis nervous tissue (n=19/20)</td>
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iso indicates iso intensity; high, high intensity; low, low intensity; sl-low, slight low intensity; sl-high, slight high intensity; and SND, selective neuronal death.

The ischemic lesion remained hyperintense on FS images as well as on T1W images in the 19 rats (Figure 3C). The 1 remaining rat had no detectable changes on serial MRI obtained 3 and 7 days after 15-minute MCAO (n=1/20, 5%) (Table). Both histological examinations at days 3 and 7 demonstrated selective neuronal death and proliferation of glial cells (reactive astrocytes and microglia) in the localized area of striatum (Table, Figures 2C, 2D, and 3G through 3J). This was consistent with the specific lesion of hyperintensity and hypointensity on T1W and T2W MRI, respectively, obtained at day 7 (Figures 3A, 3B, and 3D through 3F). This ischemic change included no infarct, hemorrhage, or lipid accumulation. No apparent calcification existed in the ischemic lesion of the striatum (Figures 2 and 3). In the rat without detectable change on repeated MRI after 15 minutes MCAO, histological examination revealed no apparent neuronal death or glial response in the caudoputamen (Table).

**Discussion**

The neuroradiological and histological data in the present study can be interpreted as follows. First, brief MCAO of 15 minutes produced no apparent MRI intensity change in the rat brain 3 days after reperfusion. Second, MRI revealed the specific ischemic change of hyperintensity and hypointensity on T1W and T2W images, respectively, in the rat striatum 7 days after reperfusion following 15 minutes MCAO. Third, this specific ischemic change on MRI histologically corresponded to selective neuronal death and proliferation of reactive astrocytes and microglia without infarct, hemorrhage, lipid accumulation, or apparent calcification. We designate this novel neuroradiological change as “delayed ischemic hyperintensity on T1W MRI” (DIH) in the following because it appeared hyperintense on T1W MRI obtained 7 days but not 3 days after reperfusion following brief MCAO.

In the present experimental study, findings on MRI similar to those in patients after spectacular shrinking deficit could be reproduced as DIH in rats subjected to 15-minute MCAO with reperfusion lasting for 7 days. Indeed, the DIH histologically proved selective neuronal death and gliosis with preservation of the total structure of the brain. The histological changes of DIH in the striatum of rats subjected to 15-minute MCAO correspond to incomplete infarction. The concept of “incomplete infarction” first described ischemic selective neuronal loss not followed by emollision in humans. Thereafter, in an experimental study, it was defined as brain lesions of selective neuronal injury produced by moderate ischemia, without panneerosis/cavitation. The neuroradiological proof of incomplete infarction has been considered possible only with use of single-photon emission CT, measuring the neuronal benzodiazepine receptor bound by radioligands, and not with conventional CT scans or MRI.

Nevertheless, an interesting question is why the selective neuronal death and gliosis in the present study appear hyperintense on T1W images and hypointense on T2W MRI. Theoretically, besides intracellular methemoglobin in hemorrhagic tissue, the following factors can shorten the T1 and T2 relaxation times: (1) factors immobilizing water molecules (macromolecular hydration effect), such as a concentrated solution of protein and calcified tissue (surface relaxation mechanism); (2) lipids; and (3) paramagnetic compounds characterized by having at least 1 unpaired orbital electron (paramagnetic proton-electron dipole-dipole interaction), including metal ions (eg, iron, manganese, copper, chromium, cobalt, and gadolinium), molecular oxygen (O2), and free radicals.

In the present study, the hyperintensity of DIH did not decline on the FS MRI. The DIH included no hemorrhage or...
lipid accumulation histologically. Additionally, the histological examination revealed no clear evidence of protein-rich solution or calcification. Additionally, 3 days after 15-minute MCAO, the histological examination revealed selective neuronal death and glial proliferation in the caudoputamen. However, the DIH had not yet appeared at that time. Considering these results together, we speculate that DIH represents some biochemical changes, such as paramagnetic species deposition that develops as tissue morphological changes progress.

In the connection with paramagnetic substance deposition in the brain, several interesting reports may support the hypothesis that the delayed ischemic hyperintensity on T1W MRI in the striatum of rats results partly from manganese or iron accumulation. Krieger et al showed that T1 hyperintensity of the globus pallidus on MRI in patients with chronic liver disease corresponded with raised tissue manganese concentrations in the globus pallidus. Free radical scavengers such as copper/zinc superoxide dismutase (CuZn-SOD) and Mn-SOD prevent neuronal damage following reperfusion after cerebral ischemia. The previous studies revealed that neurons and/or glial cells immunoreactive to Mn-SOD increased in the boundary zone of infarction in the rat striatum after 60 minutes of MCAO, in the gerbil hippocampus after 2 minutes of forebrain ischemia, and in remote brain areas after focal cortical ischemia. On the other hand, Kondo et al reported that iron accumulated in the hippocampal CA1 region, layers III–V of the parietal cortex, and frontocortical layer V in rats after 30 minutes of forebrain ischemia.

The present study indicates that conventional MRI can detect incomplete ischemic injury after mild brain ischemia as DIH: delayed ischemic change of hyperintensity and hypointensity on T1W and T2W MRI, respectively, in the striatum of rats. DIH histologically corresponded to selective neuronal death and glial proliferation; however, the specific change on MRI seems to represent some biochemical changes that affect the magnetic field as the brain tissue undergoes subtle tissue structural changes. A similar MRI pattern reported in patients who have sustained brief ischemia may represent similar histology. DIH can be interpreted to have either positive or negative significance. For example, if DIH results from induced Mn-SOD, the specific change on MRI reflects the protective system of the brain against ischemia. If DIH represents free radicals, it could be a therapeutic target after mild brain ischemia. We are investigating further the chronological change of DIH and the biochemical changes involved.

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References
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