Sequential Changes in Ischemic Edema Following Transient Focal Cerebral Ischemia in Rats: Magnetic Resonance Imaging Study

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Abstract
Sequential and regional changes in ischemic edema following various durations of focal cerebral ischemia were studied by magnetic resonance (MR) imaging in a rat unilateral intraluminal middle cerebral artery occlusion model. Occlusion was performed from 5 minutes to 5 hours. T2-weighted images were obtained chronologically 6 hours after onset of ischemia, on day 1 and day 7. An immunohistochemical study using antibodies to calcineurin and glial fibrillary acidic protein was performed to observe histological changes in the ischemic brain. The T2 high-signal-intensity areas representing ischemic edema were observed in the lateral striatum and/or the cerebral cortex by day 1 in all rats with 1- to 5-hour ischemia, and the areas were larger and detected earlier with longer durations of ischemia. In three of six rats with 15-minute ischemia and five of six rats with 30-minute ischemia, the T2 high-signal-intensity areas appeared transiently on day 1 in the dorsolateral striatum where loss of neurons expressing calcineurin immunoreactivity and associated gliosis were found. MR imaging in animal models of reversible focal ischemia can achieve sequential and noninvasive evaluation of dynamic regional changes in ischemic edema.

Key words: ischemic edema, magnetic resonance imaging, transient cerebral ischemia, striatum

Introduction
Reperfusion after cerebral arterial occlusion occurs following the spontaneous dissolution of an embolus, after fibrinolytic therapy for cerebral embolism, and after the removal of temporary clips during surgery for aneurysms or arteriovenous malformations. Determination of the temporary and regional changes in ischemic edema following various degrees of focal cerebral ischemia is important in these situations.

Magnetic resonance (MR) imaging is useful for detecting acute cerebral ischemia in clinical cases5,6,17 and various experimental models.2-4,7,11,12 Both T1 and T2 relaxation times are prolonged in brain edema induced by cerebral ischemia,23 but T2-weighted imaging possesses greater sensitivity for edema identification and localization1,12 and can detect ischemic edema within a few hours of onset.3,7,12 Clinical studies have included patients with various diseases (thrombosis and embolism), various degrees of both permanent and transient ischemia, and different time courses after ischemia. However, most previous experimental models have involved permanent ischemia in rats2,4,7 or cats.13 Although cerebral infarction in gerbils induced by the unilateral carotid artery occlusion for 60 minutes has been studied by MR imaging,3,12 the changes in ischemic edema following occlusion over time have not been fully evaluated.

We previously used a rat model of transient focal cerebral ischemia using a method of intraluminal vascular occlusion mimicking human embolic stroke13-15 to show that about 90% of rats subjected to 30 minutes of ischemia which exhibited neurological deficits during ischemia revealed characteristic histological changes including selective neuronal loss and gliosis in the striatum on the ischemic side.53 Here we report the evaluation of serial T2-weighted
MR images after vascular occlusion for various durations in this rat model.

Materials and Methods

I. Animal model
Male Wistar rats weighing 240-270 g were used in the experiments. The rats were fasted, but given water ad libitum the night before the experiment. We employed the reversible type of focal ischemia model described by Koizumi et al. and Longa et al. as modified by Nakano et al. Briefly, the right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were exposed via an anterior cervical incision under general anesthesia with a mixture of 1.5% halothane, 30% oxygen, and 70% nitrous oxide. The ECA was ligated and cut at a site 2-3 mm distal to the CCA bifurcation, and then turned upside down. An 18.5 mm length of silicone-coated 4-0 nylon thread was introduced into the ECA lumen through a small incision 1 mm distal to the bifurcation and advanced from the ECA through the ICA so that the tip of the thread reached the proximal segment of the anterior cerebral artery and obstructed the origin of the middle cerebral artery (MCA). The ECA with the thread embolus was ligated to prevent bleeding. The operative procedure usually took about 15 minutes. The maximal duration of anesthesia was set at 30 minutes in this experiment. The rectal temperature was continuously monitored with a thermometer (Digital Thermometer; Unique Medical, Tokyo) and was controlled between 37.5 and 38.0°C using a heating lamp. Restoration of ICA and MCA blood flow was achieved by removing the thread embolus under brief halothane anesthesia following various durations of ICA and MCA occlusion (5, 15, and 30 min, and 1, 3, and 5 hrs). Prior to restoration of the ICA blood flow, paresis of the left limbs and circling movement to the left were observed after discontinuing the anesthesia in the 15-minute to 5-hour ischemia groups. In the 5-minute ischemia group, paresis of the left limbs was observed just after restoration of the ICA blood flow and discontinuation of the anesthesia. Animals without limb paresis were excluded from this study. Each group comprised four to six animals for assessment of sequential changes in T2 high-signal-intensity area. Rats undergoing no surgical procedure served as the control group (n = 6).

II. MR imaging
The rats were examined using a 7.05-Tesla MR system with an 18.3-cm horizontal bore and proton frequency of 300.0 MHz (SIS 300/183; Spectroscopy Imaging Systems Corp., Fremont, Cal., U.S.A.). The rats were placed prone in a slotted tube resonator under light halothane anesthesia and T2-weighted coronal brain images were obtained with a 1.2-mm slice thickness at the striatal level, 8 mm anterior to the interaural line, using a repetition time of 3000 msec and an echo time of 100 msec on a 128 × 128 matrix.

MR images were obtained 6 hours, 24 hours (day 1), and 7 days (day 7) after the onset of ischemia. For example, the MR image at 6 hours was performed 5 hours after reperfusion in the 1-hour ischemia group, and 1 hour after reperfusion in the 5-hour ischemia group.

The T2 high-signal-intensity area was measured to evaluate ischemic brain edema by manual outlining in the cerebral cortex and striatum on the ischemic side, and computer calculation of the high-signal-intensity area as a percentage of the area of the ipsilateral hemisphere. The data is presented as the mean ± SEM. Differences in the mean T2 high-signal-intensity area between groups and times of examination were determined by the one-way analysis of variance test. A p value of <0.05 was considered to be significant.

III. Histological examination
After the MR imaging studies were completed, the rat brains were perfusion-fixed with 0.9% saline in 0.1 M phosphate buffer (pH 7.4) under deep pentobarbital anesthesia, followed by cold fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed and postfixed in the same fixative solution for 24 hours at 4°C. Immunohistochemical studies were performed on 10- or 20-μm thick vibratome sections using antibody to calcineurin and glial fibrillary acidic protein (GFAP) (Dako Corp., Carpinteria, Cal., U.S.A.). Calcineurin, a Ca++/calmodulin-regulated protein phosphatase, was used as a marker for striatal projection neurons, and the results of this study were previously described in detail. Conventional histological examination used 5-μm thick paraffin sections including the structures of the striatum, using hematoxylin and eosin and Klüver-Barrera staining.

Results
The lateral ventricles and the base of the brain in normal control rats appeared as high-signal-intensity areas on the coronal T2-weighted images at the striatal level, and the striatum was seen as a slightly
lower-signal-intensity area than the cerebral cortex (Fig. 1).

In the 5-minute ischemia group, all animals recovered from paresis of the left limbs and circling movement to the left within 30 minutes after restoration of the MCA blood flow. No abnormal signal-intensity areas were found on T2-weighted images and no histological changes were observed in the striatum or the cortex on the affected side.

A high-signal-intensity area in the lateral striatum on the ischemic side was seen at day 1 in three of six rats in the 15-minute ischemia group, and in five of six rats in the 30-minute ischemia group, although no abnormal signal-intensity areas were observed at 6 hours and on day 7 in these groups (Table 1), and recovery from paresis required a few hours. Immunohistochemical staining demonstrated a loss of calcineurin immunoreactivity accompanied with gliosis in the dorsolateral striatum in these eight rats (Fig. 2). The other rats without T2 high-signal-intensity areas in the striatum or the cortex recovered from paresis within 30 minutes, and no histological changes were seen.

A T2 high-signal-intensity area in the striatum and/or the cortex of the MCA territory was seen in three rats at 6 hours, in six rats on day 1, and in five rats on day 7 in the 1-hour ischemia group (Table 1). The mean T2 high-signal-intensity area was significantly larger on day 1 than at 6 hours or on day 7 (Table 2, Fig. 3A, D, G). The paresis continued over day 1, but had disappeared by day 7 in all rats. Histologically, loss of calcineurin immunoreactivity accompanied by gliosis was observed in the striatum and a part of the cerebral cortex in the MCA territory.

All rats in the 3- and 5-hour ischemia groups showed high-signal-intensity areas in the striatum and/or...
the cortex on the T2-weighted images at each time (Table 1). Longer durations of ischemia were associated with larger high-intensity areas (Table 2, Fig. 3B, C, E, F, H, I). Differences in size of the T2 high-signal-intensity area between 1- and 3-hour ischemia, and between 3- and 5-hour ischemia were significant at 6 hours and day 1, but not at day 7. The mean T2 high-signal-intensity areas were largest on day 1. Paresis in the left limbs was still observed on day 7, and total tissue necrosis (infarction) was seen in the lateral striatum and in the entire cortex of the MCA territory in the 3- and 5-hour ischemia groups.

**Discussion**

Our MR imaging study using the transient focal ischemia model showed that T2 high-signal-intensity areas appeared earlier and the size increased with the duration of ischemia. T2 high-signal-intensity areas demonstrating ischemic edema of the striatum and the cortex were seen at 6 hours in half of the rats in the 1-hour ischemia group and in all rats in the 3-

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<th>Duration of ischemia</th>
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Values are mean ± SEM expressed as a percentage of the area of the ipsilateral hemisphere. *p < 0.05, *p < 0.01 vs. data at 6 hours. *p < 0.02 vs. data at day 1. *p < 0.05, *p < 0.01 vs. data of 1-hour ischemia. *p < 0.05, *p < 0.01 vs. data of 3-hour ischemia.

Fig. 3 Sequential T2-weighted MR images at 6 hours (A–C), day 1 (D–F), and day 7 (G–I) in rats subjected to 1 hour (A, D, G), 3 hours (B, E, H), and 5 hours (C, F, I) of ischemia, showing that the high-intensity areas in the striatum and the cortex increased with the duration of ischemia.

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and 5-hour ischemia groups. These MR imaging findings may correspond to the "maturation" phenomenon described by Ito et al.\textsuperscript{[10]}.

Reperfusion earlier than 1 hour following the onset of ischemia significantly reduced the size of the T\textsubscript{2} high-signal-intensity area and histological ischemic damage. However, reperfusion after ischemia lasting more than 3 hours may not be beneficial because there was no significant difference in the size of the T\textsubscript{2} high-signal-intensity area at day 7 between the 3- and 5-hour ischemia groups, and total tissue necrosis was observed in the striatum and the entire neocortex of the MCA territory in both groups. These findings support the histological study of Mamezawa et al.,\textsuperscript{[15]} who investigated ischemic brain damage in rats following various durations of MCA occlusion, and found that reperfusion after 2 hours of ischemia failed to salvage penumbral tissue. Ito et al.\textsuperscript{[10]} measured the water content of ischemic brain in the Mongolian gerbil after restoration of cerebral blood flow following temporary ischemia, and found that recirculation after less than 1 hour of ischemia drastically reduced the degree of brain edema, but recirculation greatly worsened the brain edema following more than 3 hours of ischemia. These data suggest that fibrinolytic therapy for embolic stroke patients with ICA and MCA occlusion may not only be useless but may even be harmful if recirculation is obtained more than 3 hours after onset. However, these reversible focal ischemia models in rats and gerbils may produce more severe ischemic damage than in larger animals or humans because of poor collateral circulation.\textsuperscript{[9,15]}

A T\textsubscript{2} high-signal-intensity area was observed in the striatum at day 1 in some rats in the 15- and 30-minute ischemia groups, and these rats revealed histological changes in the dorsolateral striatum on day 7, when the T\textsubscript{2} high-signal-intensity area had disappeared. The transient increase in the T\textsubscript{2} signal intensity of the striatum appears to reflect a reversible focal increase in water content, although this was not measured in our study. The time threshold for the formation of ischemic cerebral edema has been studied in the baboon brain, which could withstand 30 minutes of ischemia of the MCA region without significant edema formation.\textsuperscript{[15]} However, the possibility of a delayed increase in edema fluid in the striatum cannot be excluded, as seen in the microgravimetry study of the cortex 60 minutes after reperfusion by Bell et al.\textsuperscript{[14]}

T\textsubscript{2}-weighted MR images following temporary cerebral ischemia showed that the T\textsubscript{2} high-signal-intensity areas of the striatum and the cortex were larger and occurred earlier following longer durations of ischemia. The application of MR imaging to a rat reversible ischemia model is useful to sequentially and noninvasively evaluate the regional dynamic changes in ischemic edema.

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**References**

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