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*Et al.*

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# Stroke

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**Transient and Permanent Resolution of Ischemic Lesions on Diffusion-Weighted Imaging After Brief Periods of Focal Ischemia in Rats : Correlation With Histopathology • Editorial Comment: Correlation With Histopathology**

Fuhai Li, Kai-Feng Liu, Matthew D. Silva, Tsuyoshi Omae, Christopher H. Sotak, Joseph D. Fenstermacher, Marc Fisher, Chung Y. Hsu and Weili Lin

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# Transient and Permanent Resolution of Ischemic Lesions on Diffusion-Weighted Imaging After Brief Periods of Focal Ischemia in Rats

## Correlation With Histopathology

Fuhai Li, MD; Kai-Feng Liu, MD; Matthew D. Silva, BS; Tsuyoshi Omae, MD; Christopher H. Sotak, PhD; Joseph D. Fenstermacher, PhD; Marc Fisher, MD

**Background and Purpose**—The early ischemic lesions demonstrated by diffusion-weighted imaging (DWI) are potentially reversible. The purposes of this study were to determine whether resolution of initial DWI lesions is transient or permanent after different brief periods of focal brain ischemia and to evaluate histological outcomes.

**Methods**—Sixteen rats were subjected to 10 minutes (n=7) or 30 minutes (n=7) of temporary middle cerebral artery occlusion or sham operation (n=2). DWI, perfusion-weighted imaging (PWI), and T<sub>2</sub>-weighted imaging (T<sub>2</sub>WI) were performed during occlusion; immediately after reperfusion; and at 0.5, 1.0, 1.5, 12, 24, 48, and 72 hours after reperfusion. After the last MRI study, the brains were fixed, sectioned, stained with hematoxylin and eosin, and evaluated for neuronal necrosis.

**Results**—No MRI or histological abnormalities were observed in the sham-operated rats. In both the 10-minute and 30-minute groups, the perfusion deficits and DWI hyperintensities that occurred during occlusion disappeared shortly after reperfusion. The DWI, PWI, and T<sub>2</sub>WI results remained normal thereafter in the 10-minute group, whereas secondary DWI hyperintensity and T<sub>2</sub>WI abnormalities developed at the 12-hour observation point in the 30-minute group. Histological examinations demonstrated neuronal necrosis in both groups, but the number of necrotic neurons was significantly higher in the 30-minute group (95±4%) than in the 10-minute group (17±10%, *P*<0.0001).

**Conclusions**—Transient or permanent resolution of initial DWI lesions depends on the duration of ischemia. Transient resolution of DWI lesions is associated with widespread neuronal necrosis; moreover, permanent resolution of DWI lesions does not necessarily indicate complete salvage of brain tissue from ischemic injury. (*Stroke*. 2000;31:946-954.)

**Key Words:** cerebral ischemia, focal ■ magnetic resonance imaging ■ middle cerebral artery occlusion ■ neuronal damage ■ rats

Studies in both experimental stroke models<sup>1-3</sup> and stroke patients<sup>4-8</sup> have demonstrated that diffusion-weighted imaging (DWI) is superior to conventional MRI in detecting early ischemic changes. The ischemic hyperintensity on DWI can be detected as early as 3 minutes<sup>9-11</sup> after the onset of ischemia and is due to a reduction of the apparent diffusion coefficient (ADC) of water, presumably related to water movement from the extracellular space to the intracellular spaces caused by energy failure after disturbance of blood flow.<sup>12-14</sup> The ischemic hyperintensity demonstrated by DWI is reversible if the interrupted blood flow is restored rapidly.<sup>9,10,15,16</sup> A recent study showed that resolution of DWI lesions is transient after 30 minutes of transient focal ischemia.<sup>17</sup> However, it has not been reported whether transient or

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### See Editorial Comment, page 953

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permanent resolution of DWI lesions after reperfusion depends on the duration of ischemia and whether these 2 events, if any, reflect different histopathological changes within tissue. Repeated measurements of DWI after brief periods of focal ischemia could thus provide useful information about neurological outcome and treatment strategies for stroke patients.

The goals of the present study were to investigate the time course of ischemic changes on DWI after different periods of transient, focal brain ischemia and to determine the histopathological outcomes in the regions where DWI abnormalities were permanently or transiently reversible. To ac-

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comply with this goal, diffusion-, perfusion-, and  $T_2$ -weighted MRI was repeatedly measured in the rat from acute to subacute (72 hours) time points after either 10 or 30 minutes of transient middle cerebral artery (MCA) occlusion, and histological brain tissue damage was assessed after 72 hours of reperfusion.

## Materials and Methods

### Animal Preparation

All procedures used in this study were performed in accordance with our institutional guidelines (protocol A-643). Sixteen male Sprague-Dawley rats (Taconic, Inc, Germantown, NY) weighing 300 to 340 g were anesthetized with an intraperitoneal injection of 400 mg/kg chloral hydrate. PE-50 polyethylene tubing was inserted into the left femoral artery for continuous monitoring of mean arterial blood pressure and for obtaining blood samples for the determination of pH,  $P_{aCO_2}$ , and  $P_{aO_2}$  before occlusion and at 90 minutes and 72 hours after reperfusion. Another silicone rubber tube was inserted into the left femoral vein for injection of gadopentetate dimeglumine for perfusion-weighted imaging (PWI) to estimate blood flow. During the surgical procedure, temperature was continuously monitored with a rectal probe and maintained at 37°C with a thermostatically controlled heating lamp.

### Focal Cerebral Ischemia

The intraluminal MCA suture model<sup>18</sup> was used to produce focal cerebral ischemia. Fourteen rats were subjected to either 10 or 30 minutes of transient MCA occlusion ( $n=7$  per group), and 2 other rats underwent sham operation. In all animals, the right common carotid artery, internal carotid artery, and external carotid artery were exposed through a midline incision of the neck. The proximal portions of the right common carotid artery and external carotid artery were ligated with 5-0 surgical sutures. The animal's head was then fixed in a holder with a tooth bar and ear bars.

In the 10-minute group, the rats' MCAs were occluded within the magnet unit by using an in-bore suture MCA occlusion method (described by Li and colleagues<sup>11</sup>) because of time limitations. After the occluding device consisting of supporting tubing, intra-arterial tubing, a driving line, and a piece of 4-0 silicone-coated nylon suture was set up, the rats were then placed into the bore of the magnet. Inside the magnet, anesthesia was maintained with 1.0% isoflurane delivered in air at 1.0 L/min. Body temperature was maintained at 37°C with a thermostatically regulated, heated-air flow system. Arterial occlusion was achieved within the bore of the magnet by advancing the end of the driving line until resistance was felt, indicating that the occluding filament was properly positioned in the right anterior cerebral artery and thus, that blood flow into the root of the MCA had been blocked. In the 30-minute group, a 4-0 silicone-coated nylon suture attached to a driving line within support tubing was inserted through a small incision in the right common carotid artery 3 mm below the carotid bifurcation and advanced into the internal carotid artery until resistance was felt. The rats were then quickly placed into the bore of the magnet. In the sham-operated rats, the occluding filament was inserted only 10 mm above the carotid bifurcation. Reperfusion was accomplished by gently withdrawing the occluding filament  $\approx 10$  mm while the animal was still within the magnet.

### MRI Measurements

The MRI measurements were performed in a General Electric CSI-II 2.0-T/45-cm imaging spectrometer (GE NMR Instruments, Fremont, Calif) operating at 85.56 MHz for  $^1H$  and equipped with  $\pm 20$  G/cm self-shielding gradients. Multislice, DW spin-echo/echo-planar imaging (EPI) was used to map the ADC of brain water.<sup>19</sup> Eight contiguous, coronal, 2-mm-thick slices were acquired with a field of view =  $25.6 \times 25.6$  mm<sup>2</sup>, pixel resolution =  $64 \times 64$ , repetition time (TR) = 5 seconds, echo time (TE) = 74 ms, EPI data acquisition time = 65 ms, number of excitations = 2, diffusion-sensitive-gradient

pulse width = 7 ms, and diffusion-sensitive-gradient separation time = 35 ms. The first slice was a "scout" image and was used to adjust the brain position such that the second slice started from the frontal pole of the brain. Half-sine-shaped diffusion-sensitive-gradient pulses were applied along 1 of the 3 orthogonal gradient axes ( $x$ ,  $y$ , or  $z$ ). In separate experiments, 9  $b$  values ranging from 18 to 1552 s/mm<sup>2</sup> were used to measure the ADC of water along each of the 3 diffusion-gradient directions. With the use of a linear least-squares regression, the natural logarithm of signal intensity was fit to the  $b$  values; the slope of this regression line is proportional to ADC. The mean ADC ( $ADC_{av}$ ) was calculated by averaging the 3 orthogonal ADC values on a pixel-by-pixel basis<sup>20</sup> and was used to generate ADC maps.

$T_2W$  EPI was used to perform dynamic contrast-enhanced PWI for determining cerebral perfusion.<sup>21</sup> Four contiguous, coronal, 2-mm-thick slices, which corresponded to the 4 center diffusion slices, were acquired with a field of view =  $25.6 \times 25.6$  mm<sup>2</sup> and pixel resolution =  $64 \times 64$ . A total of 40 spin-echo EPIs (TR = 900 ms, TE = 74 ms, EPI data acquisition time = 65 ms, 1 excitation) was obtained for each slice. A bolus injection of 0.25 mL of gadopentetate dimeglumine was administered after acquisition of the 15th image. The PWI data were processed to obtain an estimate of the cerebral blood flow index (CBF<sub>i</sub>) as previously described.<sup>22</sup> The change in the  $T_2$  rate,  $\Delta R_2(t)$ , was obtained from the change in signal intensity based on the following relationship:

$$(1) \quad \Delta R_2(t) = -\frac{1}{TE} \times \ln \left[ \frac{S(t)}{S_0} \right]$$

where  $S(t)$  is the signal intensity at time  $t$  during passage of the contrast agent, and  $S_0$  is the baseline value of the precontrast signal intensity. For this study, only relative changes in cerebral blood volume were evaluated, and thus, knowledge of the tissue contrast agent concentration and arterial input function was not required. The relative cerebral blood volume was determined by numerical integration of the  $\Delta R_2(t)$  versus time curve. An estimate of the vascular transit time was obtained from the first moment of the  $\Delta R_2(t)$  versus time curve. The estimates of vascular transit time and relative cerebral blood volume were used to calculate CBF<sub>i</sub> by using the equation of the central volume principle:

$$(2) \quad CBF_i = rCBV/VTT$$

where CBF<sub>i</sub> was determined for each pixel. Studies have demonstrated that the CBF<sub>i</sub> measurement is able to reflect regional blood flow changes during ischemia and after reperfusion.<sup>22,23</sup> Moreover, the CBF<sub>i</sub> ratio calculated from the 2 regions with relatively similar vascular physiology, as we used in this study, is likely to better estimate relative blood flow.<sup>24</sup>

A multislice, double spin-echo EPI pulse sequence was used to map the transverse relaxation time ( $T_2$ ) of the brain.  $T_2WI$  was achieved by varying the TE for the first echo.  $T_2$  maps were constructed from 9  $T_2W$  EPIs, with TR = 5 seconds, 4 excitations, and TE<sub>1</sub> values between 20 and 110 ms. The TE for the second echo was the same as the TE for the DWI and PWI sequences (TE<sub>2</sub> = 74 ms). This strategy ensured that the DWI, PWI, and  $T_2WI$  all contained the same EPI spatial distortions. Eight contiguous, coronal, 2-mm-thick slices, which corresponded to the 8 DWI slices, were acquired with a field of view =  $25.6 \times 25.6$  mm<sup>2</sup> and pixel resolution =  $64 \times 64$ . With the use of a linear least-squares regression, the natural logarithm of signal intensity was fit to the TE values; the slope of the best-fit line is proportional to the  $T_2$  value.

PWI,  $T_2WI$ , and DWI data were acquired before occlusion (only in the 10-minute group); during occlusion (no  $T_2WI$  in the 10-minute group because of time limitations); immediately after reperfusion; and 0.5, 1.0, 1.5, 12, 24, 48, and 72 hours after reperfusion. For the 12- to 72-hour measurements, a scout image was taken to position the rat brain such that the second slice started from the frontal pole of the brain. This strategy ensured that the brain slices obtained at different time points were well matched.

### Analysis of the Region of Interest

One region of interest, a 4×4-pixel area in the center of the ischemic lesion (lateral caudoputamen) at the level of the anterior commissure (slice 4), was chosen to measure  $ADC_{av}$ ,  $CBF_i$ , and  $T_2$  values on corresponding maps. These 3 parameters were also measured in the homologous region of the contralateral hemisphere. In addition,  $CBF_i$  values were measured in the frontoparietal cortex perfused by the anterior cerebral artery and presumed to be normal in both the ipsilateral and contralateral hemispheres. A  $CBF_i$  ratio was calculated by dividing the ipsilateral  $CBF_i$  values by the contralateral  $CBF_i$  values. The  $ADC_{av}$  and  $T_2$  values in the lateral caudoputamen of the contralateral hemisphere and the  $CBF_i$  ratio in the normal frontoparietal cortex were used as controls.

### Histopathological Evaluation

Seventy-two hours after MCA occlusion, the rats were reanesthetized by an intraperitoneal injection of chloral hydrate (400 mg/kg) and transcardially perfused with 250 mL of heparinized saline, followed by 250 mL of phosphate-buffered 4% paraformaldehyde.<sup>25</sup> The rats were decapitated and the severed heads underwent overnight fixation at 4°C in the same paraformaldehyde solution. The next day, the brains were removed from the skulls and cut into seven 2-mm-thick coronal slices starting from the frontal pole of the brain. The slices were labeled A (frontal) through G (occipital) and embedded in paraffin. Histological sections, 6 μm thick, were obtained from each paraffin block and stained with hematoxylin-eosin. One section from slice C at the level of the anterior commissure, which matched slice 4 of the  $ADC_{av}$  maps, was used for histological evaluation. A coregistration method was used to localize the same region on the histology section as on the ADC maps by using a previously described method.<sup>26</sup> In brief, a grid consisting of 5×5 squares (1.5×1.5 mm<sup>2</sup> in each square) was overlaid on the  $ADC_{av}$  maps to localize the labeled region of interest. In the same manner, the grid was then used to pinpoint the corresponding site of the labeled region of interest on the histological section. Histological images were electronically collected by using a Global Laboratory image analysis system (Data Translation Inc, Marlboro, Mass) connected to a Sony video camera interfaced with an Olympus microscope system. At high (×600) magnification, the numbers of intact and necrotic neurons were counted in 5 nonoverlapping fields in each region of interest by an investigator (K-F. L.) who was blinded to the MRI data. As previously described,<sup>25,27</sup> neurons were classified as necrotic when they exhibited pyknosis, karyorrhexis, karyolysis, cytoplasmic eosinophilia ("red neuron"), or loss of affinity for hematoxylin ("ghost neuron"). The number of necrotic neurons was divided by the total number of intact plus necrotic neurons to derive a percentage. The percentage of necrotic neurons was recorded as 100% when pannecrosis (death of all types of cells, including glia and microvessel) was observed.

### Statistical Analysis

All data are presented as the mean±SD. Statistical analyses of the physiological variables were performed by using a 2-way repeated-measures ANOVA. An unpaired *t* test was used to compare the parametric variables. A 2-tailed value of *P*<0.05 was considered significant.

## Results

### Physiological Variables

The weights of the rats in the 2 ischemic groups were not significantly different from each other (329±7 versus 320±10 g). Body temperature, mean arterial blood pressure, pH,  $Paco_2$ , and  $PaO_2$  were normal both before ischemia and throughout the reperfusion period and were not significantly different between the 2 ischemic groups (see the Table).

### Physiological Variables

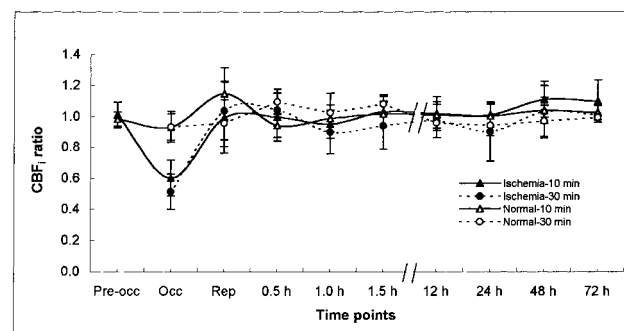
	10-Minute Group	30-Minute Group
Temperature, °C		
Baseline	37.0±0.0	37.0±0.0
90 Minutes	37.0±0.1	37.0±0.1
72 Hours	37.2±0.3	36.9±0.1
MABP, mm Hg		
Baseline	96±13	95±22
90 Minutes	105±17	96±15
72 Hours	107±13	102±17
pH		
Baseline	7.36±0.03	7.38±0.03
90 Minutes	7.35±0.03	7.38±0.03
72 Hours	7.37±0.04	7.40±0.06
$PCO_2$ , mm Hg		
Baseline	40±5	40±4
90 Minutes	36±6	38±3
72 Hours	41±6	38±4
$PO_2$ , mm Hg		
Baseline	90±7	85±5
90 Minutes	85±5	94±11
72 Hours	87±7	98±15

MABP indicates mean arterial blood pressure.

Values are mean±SD (n=7 per group). There was no significant difference between the 2 groups for all parameters (by ANOVA).

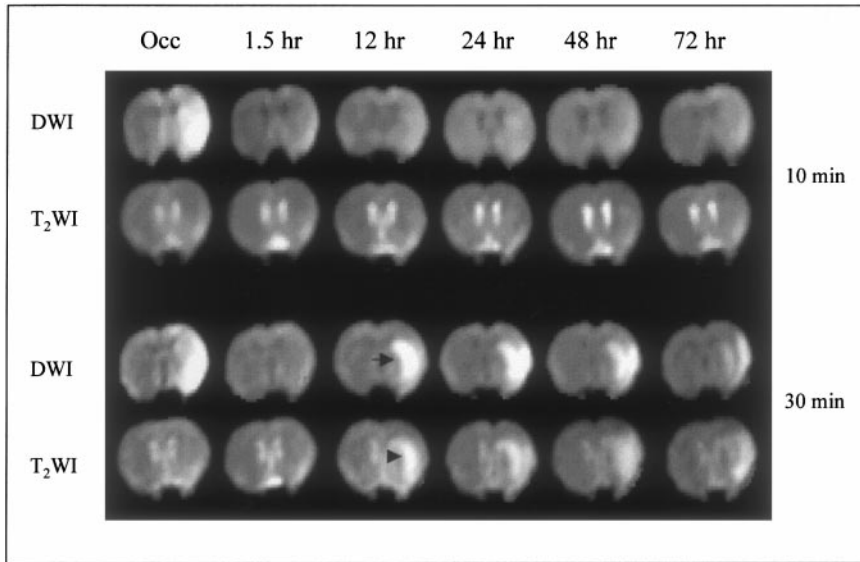
### MRI Findings

In the 2 sham-operated rats, the PWI, DWI, and  $T_2WI$  data were normal in both hemispheres throughout the period of observation. In both 10-minute and 30-minute groups, perfusion deficits as demonstrated by PWI were seen in the right MCA territory during occlusion but completely disappeared after reperfusion. Blood flow as reflected by  $CBF_i$  in the ipsilateral caudoputamen dropped to ≈50% to 60% of the



**Figure 1.** Time course of the ipsilateral cerebral blood flow ( $CBF_i$ )/contralateral  $CBF_i$  ( $CBF_i$  ratio) in the 10-minute and 30-minute occlusion groups. Blood flow in the lateral caudoputamen dropped during occlusion but fully recovered after reperfusion and remained normal up to 72 hours thereafter in both groups. Blood flow in ipsilateral normal cortex perfused by the anterior cerebral artery territory was fairly stable throughout reperfusion in both groups. Preocc, Occ, and Rep indicate before occlusion, during occlusion, and reperfusion, respectively.





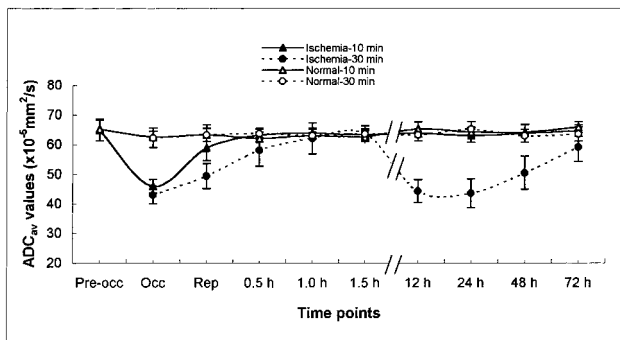
**Figure 2.** Representative diffusion-weighted images (DWI) and T<sub>2</sub>-weighted images (T<sub>2</sub>WI) in both 10-minute and 30-minute occlusion groups at different time points. DWI hyperintensity was seen during occlusion in the lateral caudoputamen and overlying cortex and completely disappeared 1.5 hours after reperfusion in both groups. The DWI and T<sub>2</sub>WI were normal thereafter in the 10-minute group, but secondary DWI lesions (arrow), accompanied by hyperintensity on T<sub>2</sub>WI (arrowhead), occurred in the 30-minute group at 12 hours after reperfusion. Note that secondary DWI lesions first developed in the caudoputamen and then gradually spread to the cortex. Occ indicates during occlusion.

contralateral flow during occlusion (Figure 1) but returned to control values (specifically, equality with the contralateral side) on reperfusion and remained normal thereafter. The drop in the CBF<sub>i</sub> ratio in the ischemic region during occlusion was significant ( $P < 0.01$ ) when compared with that in the normal region. Although it rose slightly on reperfusion, suggesting some increase in blood flow, the CBF<sub>i</sub> ratio in the frontoparietal cortex perfused by the anterior cerebral artery, the internal control region, was  $\approx 1.0$  during the entire experimental observation period (Figure 1).

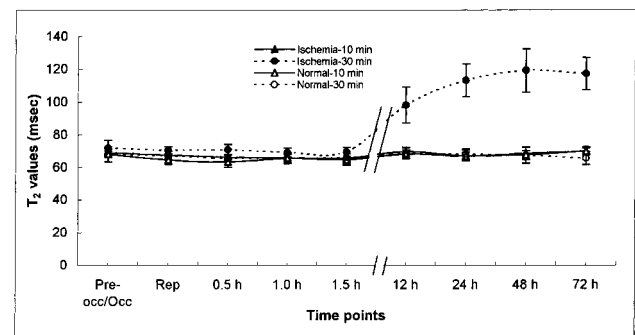
DWI hyperintensity was observed in the right MCA region during occlusion, mainly involving the lateral caudoputamen and overlying cortex (Figure 2). In the 10-minute group, DWI abnormalities in all rats gradually disappeared between 30 and 60 minutes after reperfusion, and no rats developed secondary DWI abnormalities during the 72-hour observation period after reperfusion (Figure 2). In the 30-minute group,

DWI abnormalities gradually reverted to normal between 60 and 90 minutes after reperfusion, whereas secondary DWI hyperintensities appeared at the 12-hour observation point in all rats (Figure 2). The temporal evolution of ADC<sub>av</sub> changes in both groups is shown in Figure 3. In the contralateral nonischemic hemisphere, the ADC<sub>av</sub> values in both groups were in the normal range over time ( $62$  to  $65 \times 10^{-5}$  mm<sup>2</sup>/s). In the ipsilateral ischemic hemisphere, the ADC<sub>av</sub> values decreased significantly during occlusion ( $P < 0.001$ ) compared with those in the contralateral regions but fully recovered after reperfusion in both groups. The ADC<sub>av</sub> values remained normal thereafter in the 10-minute group but declined secondarily 12 hours after reperfusion in the 30-minute group ( $P < 0.001$ ).

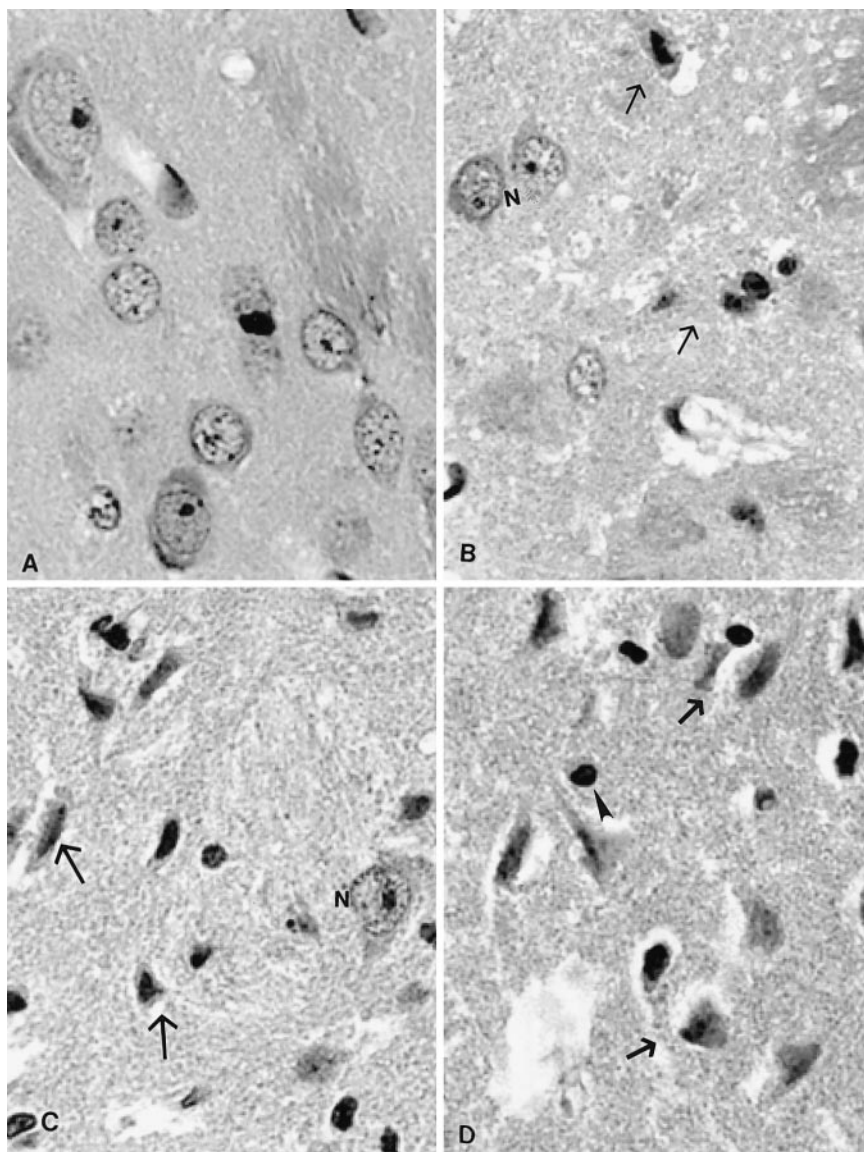
No abnormal signals on T<sub>2</sub>WI were seen in the 10-minute group, whereas hyperintensity on T<sub>2</sub>WI occurred in the 30-minute group at the 12-hour observation point (Figure 2). The changes of T<sub>2</sub> values over time are shown in Figure 4. The T<sub>2</sub> values were within the normal range in both hemi-



**Figure 3.** Time course of average apparent diffusion coefficient (ADC<sub>av</sub>) values in the ischemic and nonischemic hemispheres after 10 and 30 minutes of transient middle cerebral artery occlusion. In the contralateral (normal) caudoputamen, the ADC<sub>av</sub> values were normal. In the ipsilateral (ischemic) caudoputamen, however, the ADC<sub>av</sub> values decreased significantly ( $P < 0.001$ ) during occlusion, recovered completely at 60 minutes after reperfusion in both groups, but declined secondarily in the 30-minute group at 12 hours after reperfusion. Note that pseudonormalization of the ADC<sub>av</sub> values started at 24 to 48 hours. Preocc, Occ, and Rep indicate before occlusion, during occlusion, and reperfusion, respectively.



**Figure 4.** T<sub>2</sub> changes over time in both normal and ischemic hemispheres. In the 10-minute occlusion group, T<sub>2</sub> values were stable in both normal and ischemic hemispheres throughout the experiment, whereas in the 30-minute group, T<sub>2</sub> values in the ischemic hemisphere were significantly elevated at 12 hours and peaked at 48 hours after reperfusion ( $P < 0.005$ ). Preocc/Occ indicates before occlusion in the 10-minute group and during occlusion in the 30-minute group, respectively, and Rep indicates reperfusion.



**Figure 5.** Histological photomicrographs (hematoxylin and eosin stain, original magnification  $\times 200$ ). A, Normal appearance of brain tissue. B, Individual or isolated necrotic neurons showing pyknosis of nuclei and eosinophilia of cytoplasm (arrows) are near normal neurons (N) after 10 minutes of transient ischemia (from the same rat as in Figure 2; 10 minutes). C, Most neurons undergo necrosis (arrows) after 30 minutes of transient ischemia (from the same rat as in Figure 2; 30 minutes). Note that 1 normal neuron exists in the lesion region. D, All neurons are necrotic (arrows), and only the inflammatory cells (arrowhead) preserve normal staining patterns with hematoxylin after 30 minutes of transient ischemia.

spheres in the 10-minute group but were significantly increased ( $P < 0.005$ ) in the ipsilateral caudoputamen at 12 hours after reperfusion in the 30-minute group compared with those of the contralateral hemisphere and peaked 48 hours after reperfusion.

### Histological Outcomes

No histological abnormalities were demonstrated in the contralateral caudoputamen of the ischemic rats and in the 2 hemispheres of the sham-operated rats. Individual or isolated necrotic neurons surrounded by a microgliosis (selective neuronal necrosis) was seen in the selected region of interest in the lateral caudoputamen of the 7 rats undergoing 10 minutes of transient MCA occlusion; the proportion of necrotic neurons in this region of interest was  $17 \pm 10\%$  (range 4% to 28%). Widespread neuronal necrosis was seen in the selected region of interest of the lateral caudoputamen in 4 of the 7 rats undergoing 30 minutes of transient arterial occlusion, and pannecrosis was found in the remaining 3. The proportion of necrotic neurons was  $95 \pm 4\%$  (range 88% to

100%) in the 30-minute group and was significantly higher than in the 10-minute group ( $P < 0.0001$ ). Representative photomicrographs are shown in Figure 5.

### Discussion

We have previously demonstrated that delayed neuronal damage develops in regions where complete acute reversal of initially decreased ADC values occurs after short periods of focal ischemia (8 to 30 minutes),<sup>26</sup> and that acute normalization of ADC values induced by reperfusion is transient, and secondary ADC declines occur later on after 30 minutes of transient focal ischemia.<sup>17</sup> The present study extended our previous experiments<sup>17,26</sup> by evaluating the longer time course of DWI lesions after different short periods of ischemia and the histological status in the same regions. The novel findings in this study are (1) the resolution of initial DWI lesions is permanent after 10 minutes of transient ischemia, whereas the resolution of initial DWI lesions is transient after 30 minutes of transient ischemia, and secondary DWI lesions develop later on; and

(2) permanent reversibility of initial DWI lesions does not indicate a normal histological outcome. Conversely, selective neuronal necrosis is seen in regions where the initial DWI lesions disappear permanently after reperfusion.

### Ischemic and Postischemic Changes on PWI and DWI

PWI has been widely used to demonstrate cerebral perfusion during and after ischemia.<sup>28</sup> The  $CBF_i$  calculated from PWI data reflect relative CBF changes during ischemia and after reperfusion.<sup>22,23</sup> However, this technique may not be sufficiently sensitive to quantitatively estimate CBF reductions during ischemia, as one study demonstrated.<sup>23</sup> This observation was further confirmed by the present study, because the  $CBF_i$  ratio demonstrated only a 40% to 50% reduction, a value that is unlikely to induce substantial ischemic injury. The normalization and maintenance of a normal CBF after short periods of ischemia are in agreement with previous studies,<sup>17,26,29</sup> suggesting that postischemic injury may not be due to a secondary compromise of CBF.

DWI is able to detect ischemic changes within minutes after the onset of ischemia, and the hyperintense regions demonstrated by DWI eventually become infarcted without therapeutic intervention.<sup>1-3</sup> Evidence has shown that the ischemic hyperintensity is potentially reversible when reperfusion is performed quickly after ischemia.<sup>9,10,15,16</sup> Studies with rat stroke models indicate the dependency of this reversal on the duration of ischemia or conversely, the time of reperfusion. Reperfusion does not reduce the extent of initial DWI hyperintensity when it is performed 2 hours after focal ischemia in rats,<sup>16,30</sup> can partially reduce initial DWI lesions after 45 to 60 minutes of transient ischemia,<sup>16,30,31</sup> and can fully revert the DWI lesions within 30 minutes after the onset of ischemia.<sup>15,17,26</sup> The present study further demonstrates that complete resolution of the initial DWI lesions after reperfusion may be transient or permanent, depending on the duration of ischemia, and secondary DWI lesions may develop thereafter, accompanied by  $T_2$  abnormalities (Figure 2). Secondary DWI lesions have also been reported recently in a hypoxia-ischemia model.<sup>29</sup> Such secondary changes on DWI were thought to be caused by a delayed or secondary energy failure resulting from mitochondrial damage,<sup>32,33</sup> because ADC reduction had been shown to be closely related to reduced energy metabolism.<sup>12-14</sup>

### Tissue Damage

In the ipsilateral lateral caudoputamen 3 days after 30 minutes of transient focal ischemia, widespread neuronal necrosis was seen in 4 rats, and pannecrosis was found in the remaining 3 rats. Clearly, the secondary DWI lesions seen in this study are associated with severe brain tissue damage, and the short-term resolution of DWI lesions does not necessarily indicate tissue salvage from ischemia. This finding argues for follow-up MRI measurements after resolution of DWI lesions in the stroke patient to more completely assess tissue damage.

After 10 minutes of transient focal ischemia, selective neuronal necrosis was consistently observed in regions where blood flow, ADC, and  $T_2$  remained normal throughout the reperfusion period. Accordingly, normal blood flow, ADC,

and  $T_2$  detected by MRI after a brief period of focal ischemia may be misleading and may miss evolving tissue damage, including neuronal death. To be more specific about the latter point, the degree of selective neuronal necrosis seen on day 3 ranged from 4% to 28% of the neurons in the lateral caudoputamen, seemingly a level of tissue damage not severe enough to cause DWI and  $T_2$ WI signal abnormalities. Obviously, some subtle changes that are not detectable by current MRI measurements but lead to neuronal death are initiated after only a few minutes of markedly reduced blood flow, and even quick reperfusion cannot stop or completely reverse such processes.

Selective neuronal necrosis after a short period of focal ischemia has been documented in a previous study<sup>27</sup> and has been referred to as "incomplete infarction," because glial cells, microvessels, and tissue architecture were preserved.<sup>27,34,35</sup> Garcia and colleagues<sup>36</sup> have demonstrated that the number of necrotic neurons increases as the duration of ischemia is prolonged. Early reperfusion thus seems to shift ischemic damage from pannecrosis to incomplete infarction.

It is possible that the difference in the ability of MRI to detect tissue damage is a matter of the extent of injury and not a difference in the pathological processes between the 10-minute and 30-minute occlusion groups. The MRI data are gathered over a 2-mm-thick slice of brain tissue. The amount of tissue injury engendered by 10 minutes of transient focal ischemia may be small and become "lost" among the seemingly normal cells in that slice but become prominent enough to be detectable by MRI after 30 minutes of reduced blood flow.

### Detection of Incomplete Infarction by Other Imaging Techniques

Some imaging techniques have the potential to detect incomplete infarction. Investigations in baboons and cats in which benzodiazepine receptors were mapped by positron emission tomography have shown that an increase in peripheral-type receptor activity and a decrease in central-type receptor activity suggest selective neuronal loss indirectly and directly, respectively.<sup>37,38</sup> In a study of stroke patients, Nakagawara and colleagues<sup>39</sup> found a decrease in the central-type benzodiazepine receptor concentration in reperfused cortex that appeared structurally normal and suggested that incomplete infarction can be detected by quantifying benzodiazepine receptor activity. Because of the relatively low resolution of positron emission tomography or single-photon emission computed tomography and the low concentration of benzodiazepine receptors in the caudoputamen, the use of these imaging modalities may be limited.

Recently, Fujioka and colleagues<sup>40,41</sup> demonstrated that incomplete infarction caused by a short period of ischemia was detectable by conventional MRI after 1 week. In patients with transient hemispheric ischemia caused by cardiogenic emboli<sup>40</sup> and in rats undergoing 15 minutes of transient MCA occlusion,<sup>41</sup>  $T_1$ WI hyperintensity and  $T_2$ WI hypointensity were observed 7 days after the onset of ischemia. Selective neuronal death and gliosis with preservation of tissue structure (incomplete infarction) were seen in histological sections of rat brain from the regions that showed this combination of



delayed hyperintensity and hypointensity. Because delayed T<sub>1</sub>WI hyperintensity and T<sub>2</sub>WI hypointensity did not occur in the ischemic regions where pannecrosis was seen,<sup>41</sup> such novel signal changes on conventional MRI at delayed time points may prove to be important diagnostic signs of incomplete infarction.

### Clinical Implications

The experimental findings in this study may provide clinicians with at least 2 pieces of important information. First, complete resolution of DWI lesions has recently been reported in patients with transient ischemic attacks,<sup>42,43</sup> but the resolution of DWI lesions in some patients may be transient, as our study suggests. A series of follow-up MRI measurements may thus be required to monitor the time course of ischemic changes. Second, negative MRI (DWI and T<sub>2</sub>WI) findings after an ischemic episode may not indicate normal tissue status, because the region with the permanent resolution of DWI ischemic lesions may suffer from incomplete infarction, as demonstrated by this study. This scheme may help to explain the emergence of neurological deficits in some patients who have normal DWI results after cerebral ischemia<sup>44</sup> and cognitive deficits in some patients who experience transient ischemic attacks.<sup>45</sup>

In conclusion, the present study demonstrates that transient or permanent resolution of initial lesions documented by DWI depends on the duration of ischemia and that normal MRI (DWI and T<sub>2</sub>WI) results after short periods of focal ischemia do not necessarily indicate full tissue recovery from ischemic injury.

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## Editorial Comment

Diffusion-weighted magnetic resonance imaging (DWI), developed by Dr Michael Moseley and colleagues,<sup>1,2</sup> has now been established as an important MR sequence in detecting and assessing early ischemic lesions in basic research and clinical settings. Ischemia-induced DWI changes determined by a reduction in the apparent diffusion coefficient (ADC) are probably caused by a reduced capability of water diffusion in the ischemic region. DWI is a promising MR technique, especially if supplemented by perfusion-weighted MRI, for early detection of the ischemic brain region at risk of developing infarction.<sup>3–5</sup> The potential reversal of DWI abnormalities was noted by Moseley and colleagues in their first DWI study.<sup>1</sup> This finding raised the possibility that DWI may detect early transient ischemic lesions that do not lead to permanent tissue damage. These interesting observations may be of clinical relevance. Kidwell et al<sup>6</sup> have recently demonstrated that the DWI deficits observed during TIA could resolve completely without residual changes in follow-up imaging. In a series of studies on DWI changes in early ischemic lesions, Li and colleagues have systematically examined the pathological significance of early DWI changes. They noted that complete DWI reversal after brief ischemic insult of no more than 30 minutes was associated with delayed neuronal damage.<sup>7</sup> They further demonstrated that the permanent pathological lesion was preceded by a second wave of ADC reduction after initial complete reversal of DWI abnormality.<sup>8</sup> In the preceding

article, Li and colleagues add another novel finding. They found that reversal of DWI lesion was permanent following a very brief ischemic insult (10 minutes) but was transient with a longer period of ischemic insult (30 minutes). Thus, initial DWI abnormalities minutes after ischemic insult may not predict the pathological outcomes. The second phase of DWI abnormalities may be a signature of permanent neuronal injury after transient ischemia. These biphasic DWI changes could be detected only by conducting MR imaging early after ischemic insult and at frequent intervals for an extended period of time. Efforts are needed in clinical MR research to determine whether the novel finding of biphasic ADC reduction and the pathological significance of the second-wave DWI changes in animal stroke models also occur in the ischemic human brain.

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