

Evolving Focal Cerebral Ischemia in Cats: Spatial Correlation of Nuclear Magnetic Resonance Imaging, Cerebral Blood Flow, Tetrazolium Staining, and Histopathology

Bikash Bose, MD, Stephen C. Jones, PhD, Ronald Lorig, MD, PhD, Harry T. Friel, MS, Meredith Weinstein, MD, and John R. Little, MD

The spatial correlation of nuclear magnetic resonance imaging (NMRI) and cerebral blood flow (CBF) may improve our ability to identify ischemic brain lesions and may provide further insight into the pathophysiology of early cerebral ischemia. Eleven pentobarbital-anesthetized adult cats underwent exposure of the common carotid arteries bilaterally and the right middle cerebral artery through a transorbital approach. Baseline NMRI images were obtained with a single spin-echo, multislice technique using a 0.6-T field, 0.4-cm slice thickness, and a surface coil. Focal ischemia was produced with right middle cerebral artery occlusion and potentiated with bilateral common carotid artery ligation. Sequential NMRI studies were then performed at 1, 2, 4, 6, and 12 hours or until CBF was determined in the same cats using [14C]iodoantipyrine at either 2 (n = 2), 4 (n = 2), 6 (n = 2), or 12 (n = 1) hours after the time of occlusion. This protocol allowed temporal and spatial correlation of NMRI and CBF. Alternate 5-mm brain slices were incubated with 1% 2,3,5-triphenyltetrazolium chloride (TTC) for 45 minutes at 37-41°C and frozen in liquid Freon for later autoradiographic CBF determination. Four cats were studied only with NMRI and TTC (not CBF). The correlation between areas of increased NMRI signal intensity observed in T2-weighted images (repetition time 2,000 msec, echo time 120 msec), vital staining with TTC, low CBF, and routine histology was evaluated. During the early phase (<6 hours), T2-weighted NMRI changes were localized to the central ischemic gray matter areas, as defined in the later CBF images, with no involvement of the white matter. By the twelfth hour the NMRI changes involved the entire ischemic area including gray and white matter. The initial visible changes seen on T2-weighted NMRI are suggestive of cellular edema, and the later changes are characteristic of vasogenic edema. The spread of NMRI changes compared with the ischemic area determined from autoradiographic CBF is consistent with the previously described biphasic evolution of ischemic injury. These data suggest that T2-weighted NMRI could be used clinically to delineate areas of acute ischemic stroke. (Stroke 1988;19:28-37)

The sensitivity of nuclear magnetic resonance imaging (NMRI) to detect pathologic changes in a broad spectrum of neurologic diseases has been demonstrated. NMRI provides very accurate localization of cerebral lesions, with a sensitivity superior to that of conventional computed tomography (CT).1-4

Previous reports suggested the superiority of NMRI compared with CT in the early identification of ischemic injury2,3,7-11 and indicated that ischemic changes were promptly and sharply demarcated from surrounding nons ischemic parenchyma. T2-weighted images detect infarct and focal ischemia with high sensitivity but low specificity.12 Recent reports comparing CT and NMRI in transient ischemic attacks13 and cerebral infarction14 propose that the sensitivity of NMRI and the specificity of CT might complement each other. The investigation of experimental cerebral ischemia11,15 has indicated that spin-echo, T2-weighted NMRI can be used to detect early changes in brain water content and intracellular-extracellular ion shifts. The spatial and temporal correlation of NMRI and CBF has not been reported previously. The present study was designed to evaluate the sensitivity of NMRI in evolving focal ischemia by spatially and temporally correlating the early NMRI findings with the measurement of cerebral blood flow (CBF) using the autoradiographic technique with [14C]iodoantipyrine, electron transport chain enzyme viability (mapped with 1% 2,3,5-triphenyltetrazolium chloride [TTC]), and histopathologic changes. By using a study design based on the correlation of NMRI, autoradiographic CBF, TTC metabolic mapping, and histology in each animal, comparisons were independent of variations in the ischemic deficit between animals. To study the evolution of ischemia, animals were studied at different times after occlusion with the same modalities.

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Materials and Methods

Animal Preparation

Eleven cats, with a mean (SD) body weight of 4.2 (0.6) kg, were anesthetized with 30 mg/kg i.p. sodium pentobarbital and given 0.6 mg/kg s.c. atropine and 1.0 ml penicillin G benzathine (150,000 IU/ml) and penicillin G procaine (150,000 IU/ml). Supplemental doses of pentobarbital were given to maintain anesthesia. Cats were surgically prepared before NMRI according to previously reported procedures.\textsuperscript{16} A tracheotomy was performed, both common carotid arteries (CCAs) were exposed, and umbilical tapes were passed around them. The femoral arteries and veins were cannulated bilaterally with 16-gauge, 12-inch cutdown catheters. The right orbit was exenterated, and the right middle cerebral artery (RMCA) was exposed by the transorbital approach. The dural defect was lined with gelfoam. Incisions were closed with 4-0 Prolene suture. Arterial blood gases (Paco\textsubscript{2}, Pao\textsubscript{2}, and pH) were measured, and arterial blood pressure was continuously monitored and recorded as mean arterial blood pressure (MABP). Body temperature was maintained at 37\degree C (rectally) using an isothermal pad. One cat was prepared as a sham-occluded control: all the above procedures were performed, but the RMCA and CCAs were not occluded.

Experimental Protocol

Following exposure of the RMCA and stabilization of physiologic parameters, the cats were firmly positioned on a plastic board and baseline NMRI images were obtained. The cats were removed from the scanner, the RMCA was occluded by coagulation with bipolar cautery, and both CCAs were ligated with the previously placed umbilical tape. The protocol was designed to correlate NMRI results with other modalities. To accomplish this, different cats were imaged for 2, 4, 6, or 12 hours after RMCA occlusion (Table 1). Following the last NMRI acquisition, the cats were removed from the scanner, a CBF study was performed in seven of the 11 animals, the cats were killed, and TTC staining was performed. Table 1 outlines the different procedures and study times used for the 11 cats in this study.

The NMRI results at each time were averaged, as were the CBF results obtained at different times. In each cat, the preocclusion and postocclusion NMRI results were compared with the other parameters obtained at the end of the study. NMRI intensities from a total of eight cats were grouped, as were CBF values from a total of five cats. Cats 9 and 10 (with striate ischemia) were not included in the numerical analysis but were used for descriptive correlation. Cat 11 was analyzed as a sham-occluded control.

Nuclear Magnetic Resonance Imaging

Images were produced with a single Carr-Purcell-Meiboom-Gill spin-echo, multislice technique using a 0.6-T field, 0.4-cm slice thickness, and a surface coil placed parallel to the \(x-z\) axis of the magnet core and centered over the vertex of the skull. In all studies, T2-weighted images were acquired with repetition time (TR) 2,000 msec and echo time (TE) 120 msec. In a limited number of studies, spin-density images (TR 2,000 msec, TE 32 msec) and T1-weighted images (TR 500 msec, TE 32 msec) were obtained for cerebrospinal fluid and anatomic reference. Sequential NMRI was performed before and 1, 2, 4, 6, and 12 hours after bilateral CCA and RMCA occlusion or sham occlusion or until the termination of the study. The study times were 2 (2 cats), 4 (2 cats), 6 (3 cats, including the 1 sham-occluded control), and 12 (4 cats) hours.

NMRI data were expressed as the right-to-left (ipsilateral:contralateral) ratio of the relative intensity recorded in homologous regions of interest. Ratios obtained in this way express the difference between the right and left sides and provide a quantitative measure of the visual comparison of side-to-side intensity.

Table 1. Experimental Protocol

<table>
<thead>
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<th>Cat no.</th>
<th>Final time (hr)</th>
<th>Modality</th>
<th>Light microscopy</th>
<th>Comments</th>
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<td></td>
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<td>NMRI</td>
<td>CBF</td>
<td>TTC</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>x</td>
<td>x</td>
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<td>11</td>
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NMRI, nuclear magnetic resonance imaging; CBF, cerebral blood flow determination; TTC, 2,3,5-triphenyltetrazolium chloride vital staining.
Local Cerebral Blood Flow Determination

CBF was determined at 2 (2 cats), 4 (2 cats), 6 (1 cat), and 12 (1 cat) hours after the onset of ischemia and at 6 hours after the sham occlusion (1 cat). [14C]lodoantipyrine ([14C]IAP, 250 μCi, specific activity 4.7 mCi/μmol, New England Nuclear, Boston, Massachusetts) was continuously infused intravenously for 45 seconds. During the infusion, timed arterial blood samples were taken at 2–5-second intervals for the determination of CBF.17,18 The [14C] activity in these samples was measured with a liquid scintillation counter (Beckman, Fullerton, California). The cats were killed at the end of the [14C]IAP infusion by a rapid intravenous injection of saturated KCl. The brains were removed and rapidly cut into 5-mm coronal sections. Alternate 5-mm sections were frozen in Freon-22 (Du Pont, Wilmington, Delaware); 20-μm sections were cut from these 5-mm sections in a cryostat at —20°C, thaw-mounted on glass coverslips, and dried at 60°C on a hotplate. The sections were then placed on x-ray film (SB-5, Kodak, Rochester, New York) for 7 days with calibrated [14C]-labeled methylmethacrylate standards (Amersham Corp., Arlington Heights, Illinois). Regional [14C] brain concentration and CBF were evaluated using a digital image processing system.19 CBF was calculated using the method of Reivich et al17 and Sakurada et al18 from the time-history of the arterial [14C] samples and the [14C] brain concentration.

Tetrazolium Mapping of Ischemic Area

Alternate 5-mm brain sections were incubated with 1% TTC (Fisher, Fair Lawn, New Jersey) for 45 minutes at 37–41°C as described previously.20,21 Brain slices were immersed in 4% buffered formalin and photographed to record the degree and extent of TTC staining. Normal areas appear red (black in Figure 2), whereas transitional or penumbra ischemic zones are pink (gray), and totally ischemic areas (which lack the electron transport chain enzymes) appear white.

Light Microscopy

After 2 weeks of immersion-fixation in 4% buffered formalin, the TTC-reacted sections were stained with hematoxylin and eosin. Sections were examined for ischemic cell change according to the grading scheme of Little22,23: slight (Grade I), moderate (Grade II), and severe (Grade III) neuronal alterations.

Data Selection and Region Identification

Four parameters (NMRI intensity, CBF, amount of TTC staining, and histology) were available for qualitative and quantitative analysis. So the data could be correlated among cats over the range and extent of ischemia from the center of the ischemic zone to the normal cortex, the most severely affected pair of CBF and TTC images were chosen from adjacent 5-mm brain slices. The NMRI image nearest the other two images was then selected. The NMRI images appear intense in different areas than the TTC and CBF images; CSF in the ventricular spaces is bright in T2-weighted images,7 while in the TTC and CBF images the ventricular spaces have collapsed because the brain is ex situ.

NMRI absolute signal intensities and CBF values were analyzed in three regions on both the side contralateral to the occlusion and the ipsilateral (right) side. Region selection was based on the location of the ischemic area for each cat, defined unambiguously by the CBF autoradiogram. This procedure allowed a consistent comparison of the NMRI, CBF, and TTC images and histology, even though there were variations in the location and size of the ischemic area among cats. Selected regions included deep gray matter (either basal ganglia or thalamus depending on the level of the section), cortex at the ventrolateral margin (frontal or temporal lobe), and the ectosylvian area in the dorsolateral cortex (usually the middle ectosylvian gyrus) (Figure 1).

Results

Physiologic Variables

Table 2 lists the physiologic variables at the end of the NMRI study and, in those cats that were also studied using CBF, just before the CBF study. MABP is within normal limits, Pao2 is above the level at which CBF responds to hypoxia, the variation in Paco2 is minimal so CBF variability due to changes in Paco2 would not be expected, and pH is normal. The variation in Pao2 and Paco2 is due to the uncontrolled respiration of these cats and is expected in pentobarbital-anesthetized, nonventilated animals.

Local Cerebral Blood Flow

The mean CBF in each area sampled is given in Table 3. Because these areas were chosen from the CBF images to represent the most consistent areas of ischemia, the exact location of the ischemic zone was different for different cats.

The consistent and severe decreases in CBF in the ectosylvian and temporal regions of interest ipsilateral to the MCA occlusion are documented by the ipsilateral:contralateral (I:Q) ratios of 0.06 and 0.05 in these areas. Low CBF was observed in these regions regardless of the duration of ischemia; CBF values were

![Regions of interest for quantitative analysis of nuclear magnetic resonance image intensity and cerebral blood flow (ectosylvian, temporal [ventrolateral] cortex, and deep gray regions) were chosen on basis of the position of the ischemic focus for each cat.](https://stroke.ahajournals.org/)

Figure 1.Regions of interest for quantitative analysis of nuclear magnetic resonance image intensity and cerebral blood flow (ectosylvian, temporal [ventrolateral] cortex, and deep gray regions) were chosen on basis of the position of the ischemic focus for each cat.
pooled for comparison of I:C ratios between regions. Relative hyperemia (I:C ratio 1.8) was observed in the ipsilateral deep gray region. The ipsilateral flows in the ectosylvian and temporal (3.0 and 2.1 ml/min/100 g) areas are well below the value of 20 ml/min/100 g normally accepted as the critical level of flow for an infarct to occur.24 For the sham-occluded cat, the CBF pattern was symmetric (Table 3). The control CBF values compare well with the contralateral CBFS from the experimental cats.

The CBF autoradiograph of Cat 5 (6 hours) is shown in Panel D of Figure 2. Both the extent and level of ischemia can be compared with NMRI intensity and TTC staining in this figure. There were three patterns of ischemia in the cats studied. In one group, the ischemic area does not include the deep gray area (thalamus) and occupies approximately 50% of one hemisphere, but hyperemia is present in the thalamus. Panel B of Figure 2 is representative of another group in which the entire hemisphere was ischemic, while in a third group only the lenticulo-striate vascular network was affected. In general, the area of ischemia produced by the combination of MCA and bilateral CCA occlusion was larger than that normally observed using MCA occlusion without CCA occlusion.

Tetrazolium Vital Staining

The TTC-stained section of the brain corresponding to the CBF autoradiograph is shown in Panel C of Figure 2, 6 hours after occlusion. There is a high correlation between TTC staining and the CBF autoradiograph, especially after 4 hours. TTC staining was normal in the sham-occluded cat.

Light areas in the TTC images indicate the absence of enzymes of the electron transport chain23 and correlate well with low-flow areas as shown in the CBF images. Dark areas in Figure 2 indicate tissue viability, with a high concentration of enzymes that have reacted to produce red formazan compounds, and correspond to high- and normal-flow areas. Intermediately stained areas, which appear pink in freshly stained tissue and gray in black and white photographs, appear either at the edge of the ischemic area or at intermediate times between the initial flow deficit and the eventual ischemic cell change.

By comparing the TTC and CBF images from the earliest (2-hour) study with the latest (12-hour), it becomes clear that the TTC pattern does not initially correspond to the CBF pattern. At 2 hours, TTC staining in the ischemic ectosylvian area indicates that most of the tissue is still viable. At 4 hours after MCA occlusion, immediately stained tissue appears in areas of intermediate flow at the ventrolateral border and at the anterior cerebral artery and MCA border territory. By 6 hours (Figure 2) and 12 hours, there is complete correspondence of TTC and CBF images; the lack of formazan compounds and flow deficit match.

Nuclear Magnetic Resonance Imaging

As shown by T2-weighted NMRI, initial changes in evolving infarction are localized to cortical areas and are correlated with the center of the ischemic area as identified by the CBF image and TTC stain. Following 6 hours of severe ischemia the changes also involve the white matter in the distribution of the RMCA (Figure 2). There is good correlation between areas of increased signal intensity during T2-weighted NMRI (TR 2,000 msec, TE 120 msec), vital staining with TTC, and CBF.

The time courses of the T2-weighted NMRI changes are shown in Figure 3. The T2 signal intensity increased linearly in the ischemic hemisphere. For the first 6 hours, progressively increasing T2 changes are seen predominantly in the ectosylvian area. Following this period the temporal and deep gray areas showed rapid increases in signal intensity. The early increase in the NMRI signal intensity ratio in the ectosylvian area is readily observed in Figure 3.

As the time course changes in Figure 3 show, by 6 hours the T2 intensity ratio in the ectosylvian region reached a plateau, while the T2 intensity in the temporal and deep gray regions had just started to increase. By 12 hours, all three areas sampled for numeric results showed increased NMRI T2 intensity. In comparing the images visually, the spread of the T2 intensity is apparent from the ectosylvian area to the more ventral region of the temporal area. In Figure 2, Panel B (Cat 5, 6 hours), note the increased intensity in the sulcus between the middle ectosylvian gyrus and the suprasylvian gyrus, highlighted by the leftmost arrow, in addition to the increased signal from the middle ectosylvian gyrus itself, highlighted by the second arrow. In Figure 4 (Cat 7, 12 hours), baseline spin density (Panel A, gray=white matter differentiation) and T2-weighted images (Panel B) are compared with 12-hour T2-weighted images (Panel C). The increase in T2-

### Table 2. Physiologic Data

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<th>No. cats</th>
<th>Mean</th>
<th>SEM</th>
<th>MABP (mm Hg)</th>
<th>PaO₂ (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
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<td>9</td>
<td>84</td>
<td>33</td>
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MABP, mean arterial blood pressure.

Blood gases and MABP (pH) were not obtained in 2 (3) of the 11 cats because of equipment malfunction.

### Table 3. Cerebral Blood Flow and Ipsilateral-Contralateral Ratio After Occlusion of MCA and CCA or Sham Occlusion

<table>
<thead>
<tr>
<th>Region</th>
<th>Ectosylvian</th>
<th>Temporal</th>
<th>Deep gray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipsilateral</td>
<td>3.0</td>
<td>2.1</td>
<td>110</td>
</tr>
<tr>
<td>Contralateral</td>
<td>49</td>
<td>40</td>
<td>66</td>
</tr>
<tr>
<td>Sham (mean of both)</td>
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<td>39</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Ratio</th>
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<th>After sham occlusion</th>
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<tr>
<td></td>
<td>0.06</td>
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<tr>
<td></td>
<td>1.8</td>
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</table>

Mean values (ml/min/100 g) from 5 cats at 2 (n = 2), 4 (n = 2), or 6 (n = 1) hours after occlusion.

MCA, middle cerebral artery; CCA, common carotid artery.
weighted intensity over the entire gray and underlying white matter of the ectosylvian and temporal regions is apparent in Panel C.

Clearly visualized abnormalities are seen only 4–6 hours after the onset of ischemia with T2-weighted images. Also, the initial changes on NMRI-weighted images in the ectosylvian area of the ischemic focus. Though changes in T2 signal intensity were seen in as soon as 1 hour, changes were detectable more often, either visually or from an inspection of the NMRI intensities, after 6 hours or longer.

**Light Microscopy**

At 2 hours, no generalized pathologic changes were noted; isolated ischemic neurons were localized to the core of ischemia in the ectosylvian area. At 4–6 hours, marked vacuolation of the neuropil was seen at the center of the ischemic zone. The changes in the neuropil were initially localized to the perivascular areas (2–6 hours); at 12 hours, the entire ischemic area was edematous with Grade III neuronal changes. There was good correlation between TTC staining and light microscopy findings after 6 hours. These changes are entirely consistent with the previously reported pattern of histopathologic changes in focal cerebral ischemia from this laboratory.

**Image Correlation and Summary**

In summary, T2 weighting, compared with other imaging sequences, demonstrated the greatest sensitivity for the detection of focal cerebral ischemia. The earliest changes at 2 hours are not visually impressive but become clearly demonstrated 6 hours after MCA occlusion. Twelve hours after MCA occlusion, increased signal intensity characterizes a widespread area including the central ischemic area and the surrounding border (Figure 4). This area includes all regions analyzed numerically. The increase in NMRI signal intensity, expressed as the ratio of the ipsilateral to contralateral values, is initially seen in the central

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**Figure 2.** A: Baseline, preoclusion and B: 6-hour postocclusion T2-weighted nuclear magnetic resonance images (NMRI) of Cat 5. Note increase (arrows) in T2-weighted intensity on side ipsilateral to middle cerebral artery occlusion in ectosylvian gyrus and sulcus immediately medial compared with contralateral side and with baseline (control) preocclusion image. 6-hour image (Panel B) does not yet show extensive NMRI changes in the ischemic zone that are later revealed in 12-hour studies. C: Slice stained with 2,3,5-triphenyltetrazolium chloride (TTC) and D: Cerebral blood flow (CBF) autoradiogram from adjacent slice. Extensive ischemic area is clearly shown by excellent correlation of CBF autoradiogram (Panel D) and TTC image (Panel C) and corresponds to increase in T2 intensity in third gyrus from midline (middle ectosylvian gyrus), which is core of the most severely affected area in CBF image. At this early time, absence of red formazan staining in ischemic area of TTC image indicates that progression of normal tissue to infarcted tissue has been initiated. This transition did not occur in cats studied at 2 hours.
ischemic area and in time spreads to the surrounding areas including white and gray matter.

Table 4 summarizes the spatial correlation in different cats for NMRI, CBF, and TTC. Spatial correlation between CBF and TTC images is maximal after 4 hours, while NMRI does not fully correspond with CBF or TTC until 12 hours. Table 5 summarizes the temporal changes of each modality. The temporal pattern is finalized at 12 hours when all modalities used in this correlative study show a similar spatial pattern. The dynamic pattern is initiated with changes in CBF, which occur within several minutes of the MCA occlusion; between 2 and 4 hours, the TTC changes are fully expressed, while at 12 hours the increase in NMRI intensity appears to be still progressing but has filled the area originally defined by the flow deficit. Histologic changes were fully expressed at 12 hours.

Discussion

Introduction

Early studies of cerebral ischemia and brain infarction using NMRI helped define the best series of pulse sequences to detect early cerebral ischemia. These studies documented changes in T1 and T2, although T2 changes consistently occurred before the earliest changes in T1. NMRI has been found to indicate changes as early as 1½-2 hours after experimental ischemia. Studies using tissue excised from infarcted animals and in vitro measurement of T1 and T2 indicate that the early T2 NMRI changes were due to cerebral edema. Increased water content in areas of ischemia have been noted as early as 30 minutes and 2 hours after ischemic insult.

![Figure 3](http://stroke.ahajournals.org/)

Figure 3. A: Mean T2-weighted nuclear magnetic resonance image (NMRI) right-to-left (ipsilateral:contralateral) intensity ratio in 3 areas showing increase in ectosylvian area up to 6 hours and rise in temporal and deep gray areas after 6 hours. At 0, 1, 2, 4, 6, and 12 hours there were 8, 6, 6, 4, 2, and 2 cats. Error bars represent plus or minus SEM, or for 2 observations, plus or minus range. B: Lack of change in intensity ratio of sham-occluded cat over 6 hours.

![Figure 4](http://stroke.ahajournals.org/)

Figure 4. Nuclear magnetic resonance images of Cat 7 demonstrate spread of T2-weighted intensity from gray into white matter 12 hours after occlusion. A: Spin-density image (repetition time 2,000 msec, echo time 32 msec) in which gray and white matter can be differentiated. B: Baseline and C: 12-hour postocclusion T2-weighted images. Increase in T2-weighted intensity (Panel C) is clearly visible in right cortex and white matter.
The main thrust of our study was to correlate NMRI in ischemic stroke with local CBF, tissue metabolism, and morphologic changes. Our data demonstrate that, in the initial stages of focal cerebral ischemia, NMRI changes are visible only on the spin-echo, T2-weighted images and not on spin-density or T1-weighted images. The initial T2-weighted changes are localized to the gray matter areas of the ischemic zone. Later, more diffuse changes in the T2-weighted images are seen in the entire hemisphere and involve both gray and white matter, as demonstrated in Figure 4. As T2-weighted changes appear in white matter, the second stage of ischemic edema is entered. The implication this has on the future treatment or therapeutic intervention of stroke remains to be seen. By comparing the superior anatomic detail and gray-white differentiation of the spin-density images with the sensitivity of the T2-weighted images, the severity and extent of ischemia can possibly be assessed.

Initial NMRI changes often occurred in the sulcus medial to the middle ectosylvian gyrus (between the suprasylvian and middle ectosylvian gyri). These T2 intensity increases were shown not to be attributable to an increase in sulcal width as confirmed by comparing the T2 images with a limited number of spin-density images. There was no cerebrospinal fluid-dependent increase in intensity in the area that showed increased T2 signal. This is consistent with the mass effect of ischemic edema; any potential sulcal spaces would be closed by this mechanism.

Cerebral Blood Flow in Focal Cerebral Ischemia

Bilateral CCA occlusion in addition to unilateral MCA occlusion was used in this correlative study of four modalities to increase the chance of producing a large stroke and to make the correlation between three different imaging techniques and histopathologic grading easier. This model of focal ischemia has been shown to produce consistently large areas of ischemia and infarction. Schuier et al reported that bilateral CCA occlusion combined with MCA occlusion increased the severity and extent of ischemia in the ipsilateral cortex. The severity of the ischemia (to <5 ml/min/100 g) in the ischemic areas analyzed quantitatively in this study was increased by the combined bilateral CCA and RMCA occlusion.

Our CBF values agree well with those obtained by other investigators in the cat MCA occlusion model. Cortical CBFs of 39 ml/min/100 g with 30 mg/kg pentobarbital, 45 ml/min/100 g using 1–2% halothane anesthesia, and 40 ml/min/100 g in caudate nucleus with 70% nitrous oxide anesthesia have been reported.

Relative hyperemia in the ipsilateral deep gray region was observed in this study; the ipsilateral:contralateral ratio was 1.8 in this region. This pattern of hyperemia has also been observed at the edge of the ischemic region in the basal ganglia in the rat MCA occlusion model. This hyperemia is possibly due to the border of the ischemic area being supplied by the posterior circulation.

In two cats (9 and 10), the ischemic areas were limited to the distribution of the lenticulo-striate penetrating arteries. In these cats, the CBF and the TTC results correlated well with the NMRI results and are consistent with the other cats in which the ischemic deficit was more widespread.

Tetrazolium Vital Staining

TTC is a colorless solution. TTC staining depends on the viability of enzymes of the electron transport chain; as such, it represents a variable that could be interpreted as a measure of tissue viability. When incubated with metabolically viable tissue, TTC is converted to a red formazan compound. Thus, normal tissue is stained red, while marginally ischemic areas or areas that are very ischemic but not yet infarcted are light pink, and completely infarcted areas appear white. The onset of TTC changes does not correspond to the 4–6-minute store of immediately available energy metabolites. Initial changes in TTC staining occur

<table>
<thead>
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TTC, 2,3,5-triphenyltetrazolium chloride vital staining; NMRI, T2-weighted nuclear magnetic resonance images. +, changes present (Grade III for histopathology); −/+ , intermediate or mixed changes (Grades I and II for histopathology); − , no change present.
Nuclear Magnetic Resonance Imaging Changes in Focal Cerebral Ischemia

NMRI has been used in many animal models of focal cerebral ischemia to document and define the imaging parameters most useful for clinical evaluation and to study their relation to cerebral edema. Buonanno et al.33 studied focal ischemia in rats, gerbils, and cats using NMRI with the steady-state free precession pulse sequence and in vitro spectroscopy. They reported that the earliest changes in NMRI parameters, which were determined in vitro, were noted 2 hours after ischemia and that the infarcted hemisphere had significantly prolonged T1 and T2 when compared with the contralateral hemisphere or control animals. Our results did not show changes in the T1-weighted images that were determined in vitro and our in vivo T2- and T1-weighted images.

Studies in baboons have shown that NMRI changes can be seen as soon as 90 minutes using T1-weighted images10 and are quite apparent by 5 hours as increased signal intensity using spin-echo, T2-weighted images, and decreased signal intensity on inversion recovery, T1-weighted images.11 Our findings are slightly different in that we did not find early (90 minutes) or late changes in the T1-weighted images. Our T2-weighted results do agree with their initial results obtained at 5 hours, although in our initial scans 1 hour after the ischemic insult a definite increase in T2 signal intensity was noted in some cats on the ipsilateral side. At 12 hours the NMRI changes were clearly visible.

Our results of increased T2-weighted intensities agree with the results of Kato et al.12,13 who showed that in experimental focal cerebral ischemia in gerbils and rats, T2-weighted images were superior to T1, saturation-recovery images. In addition, these investigators correlated increased T2 intensity with other retrospectively determined parameters that were indicative of cerebral edema. The biphasic evolution of edema was hypothesized to account for the spread of increased T2 intensity from the early (2–5 hours) to the later times (19 hours to 2 days). T1 and T2 measurements correlated extremely well with water content (r = 0.987 and 0.967, respectively). Our T1-weighted parameters of TR = 500 msec and TE = 32 msec did not give the contrast that theirs did (T1 = 300 msec, TR = 1,600 msec, TE = 14 msec). The left-to-right (ischemic: normal) ratios of T1 and T2 reached 1.10 and 1.13 4 hours after carotid ligation in four animals.36 In another study using unilateral CCA ligation in gerbils, 1.47- and 1.30-fold increases in T1 and T2 were noted at 24 hours.8,37 These results of other investigators correspond to our results of an ipsilateral:contralateral T2-weighted intensity ratio of 1.27 at 4 hours and 1.42 at 12 hours after focal cerebral ischemia from MCA occlusion in cats.

The early changes seen in NMRI in both experimental and clinical studies1,7,11,38-40 of cerebral ischemia are most often associated with increased water content of tissue. This parameter is not visualized using CT. Only as early changes in water content are reflected in blood–brain barrier dysfunction are CT changes noted due to enhancement.

Correlation Between Tetrazolium Staining, Cerebral Blood Flow, Nuclear Magnetic Resonance Imaging, and Histopathology

Tables 4 and 5 summarize the spatial and temporal correlations of the different modalities used in this study. The CBF decrease in the ipsilateral hemisphere in this model of MCA occlusion in cats is apparent at the earliest time in this study, 2 hours, and is the first and causative factor in this occlusive stroke model (see Table 3). TTC and NMRI changes occur next; their occurrence together could be causally related through the effect of edema. The increase and spread in T2 intensity occur during the secondary stage of cerebral ischemic edema, vasogenic edema. The lack of TTC staining depends on the loss of enzymes of the electron transport chain due to the biochemical and morphologic changes brought on by this edema. Histopathologic changes that were noted were consistent with our previous observations and reflect the late changes in cellular structure that occur 6–12 hours after occlusion. These changes are consistent with the spread of cytotoxic edema from metabolically more active gray matter to white matter and coincide with the second (vasogenic) stage of ischemic edema.

Conclusion

Our data indicate that spin-echo, T2-weighted imaging can detect early changes in focal cerebral ischemia. One cat showed changes as early as 60 minutes after ischemia. The initial NMRI changes were localized to the temporal and ectorctolin areas in the ischemic cortex and later (6–12 hours) involved deeper structures in the ipsilateral hemisphere. This initial, localized change in the NMRI images did not correspond to the CBF studies, which showed an immediate and consistent drop in blood flow in the distribution of the MCA. The correlation of NMRI with CBF studies and TTC vital staining of enzymes important in oxidative metabolism, though not great initially, were excellent with the passage of time. The increase in T2-weighted signal intensity over time and its anatomic distribution corresponded to the biphasic evolution of edema in focal cerebral ischemia that has been previously described by other workers.24,25

Our data also suggest that once NMRI changes are seen, the neural tissue may have sustained irreversible ischemic changes. The choice of various NMRI parameters of proton density and relaxation times to image different aspects of morphology, physiology, and...
tissue water content may be of help in the early differentiation between potentially viable tissue and areas that will become infarcted. In summary, T2-weighted NMRI provides early ischemic zone definition, correlates with CBF and TTC staining at later times, and is consistent with biphasic evolution of ischemic edema.

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**KEY WORDS** • cerebral ischemia • nuclear magnetic resonance imaging • pathology • cats
Evolving focal cerebral ischemia in cats: spatial correlation of nuclear magnetic resonance imaging, cerebral blood flow, tetrazolium staining, and histopathology.

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