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M Brant-Zawadzki, B Pereira, P Weinstein, S Moore, W Kucharczyk, I Berry, M McNamara and N Derugin

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MR Imaging of Acute Experimental Ischemia in Cats

Michael Brant-Zawadzki[1]
Brian Pereira[2]
Philip Weinstein[2]
Sheila Moore[1]
Walter Kucharczyk[1,3]
Isabelle Berry[1,4]
Michael McNamara[1]
Nikita Derugin[1]

The evolution of acute cerebral ischemia was documented by magnetic resonance (MR) imaging in 13 mongrel cats with occlusion of the middle cerebral artery through a transorbital approach. The animals were imaged under anesthesia at intervals from 30 min to 10 days after production of the lesion. An MR imager operating at 0.35 T was used with multislice, multi-spin-echo technique (TR = 500–2000 msec; TE = 28, 56 msec). The animals were sacrificed after imaging for pathologic correlation. Infarcts beyond 4 hr of age were visualized in all subjects. The earliest infarct was seen at 30 min (two cats) as an area of high signal intensity on T2-weighted images. In three other cats, however, 3-hr-old infarcts were not detectable. In one animal, a hemorrhage within a 1-week-old area of infarction was not characterized by MR imaging but was identified on CT scanning. The mass effect of the infarction appeared greatest at 2–4 days after infarction. The basal ganglia showed ischemic effects to best advantage. MR imaging offers previously unavailable sensitivity for the early noninvasive detection of cerebral ischemia in vivo.

The sensitivity of proton magnetic resonance (MR) imaging to cerebral edema allows superior detection of pathology in the central nervous system [1–4]. This capability of MR imaging has important implications for the experimental and clinical evaluation of acute cerebral ischemia [5–11]. Of major interest is the detection of ischemia in the earliest stages, because experimental reversibility of ischemic neurologic damage has been reported with reperfusion of the lesion in its incipient stages [12]. Indeed, some have advocated surgical correction of compromised cervical vessels in patients with acute ischemic disease of a few hours' duration [13]. On the other hand, such surgery worsens the prognosis of patients in whom irreversible infarction is present [14]. Questions that arise in this context are, How early can the changes of ischemia be seen with MR imaging? What do they represent? and, Do the earliest detected changes correlate with irreversible damage? The answers to these general questions might provide the neuroscientist and clinician with a unique tool that—unlike those used in previous experimental monitoring of acute ischemia—can be applied pragmatically both in the laboratory and in the clinical setting, and is noninvasive. To this end, we have begun to explore the usefulness of MR imaging in the evaluation of acute, potentially reversible experimental ischemia in a controlled, experimental model.

Subjects, Materials, and Methods

Thirteen adult mongrel cats underwent occlusion of the proximal middle cerebral artery (MCA) using a transorbital surgical approach. Under endotracheal anesthesia (isoflurane), the head was placed in a stereotaxic holder and the proximal MCA isolated under an operating microscope after enucleation and enlargement of the orbital foramen. Permanent occlusion was achieved with a bipolar coagulator in cats to be studied for ischemia of 12 or more hours' duration. More acute ischemia was created as follows: A titanium-reinforced, balloon occluding device (Heyer-Schulte) was gently positioned circumferentially around the MCA just past its

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[1] Department of Radiology, University of California, San Francisco, CA 94143. Address reprint requests to M. Brant-Zawadzki.

[2] Department of Neurosurgery, Veterans Administration Medical Center, San Francisco, CA 94121, and University of California, San Francisco, CA 94143.

[3] Present address: Department of Radiology, University of Toronto, Toronto, Ontario, Canada.

[4] Present address: Service de Neuroradiologie, Hôpital Purpan, Toulouse, France.

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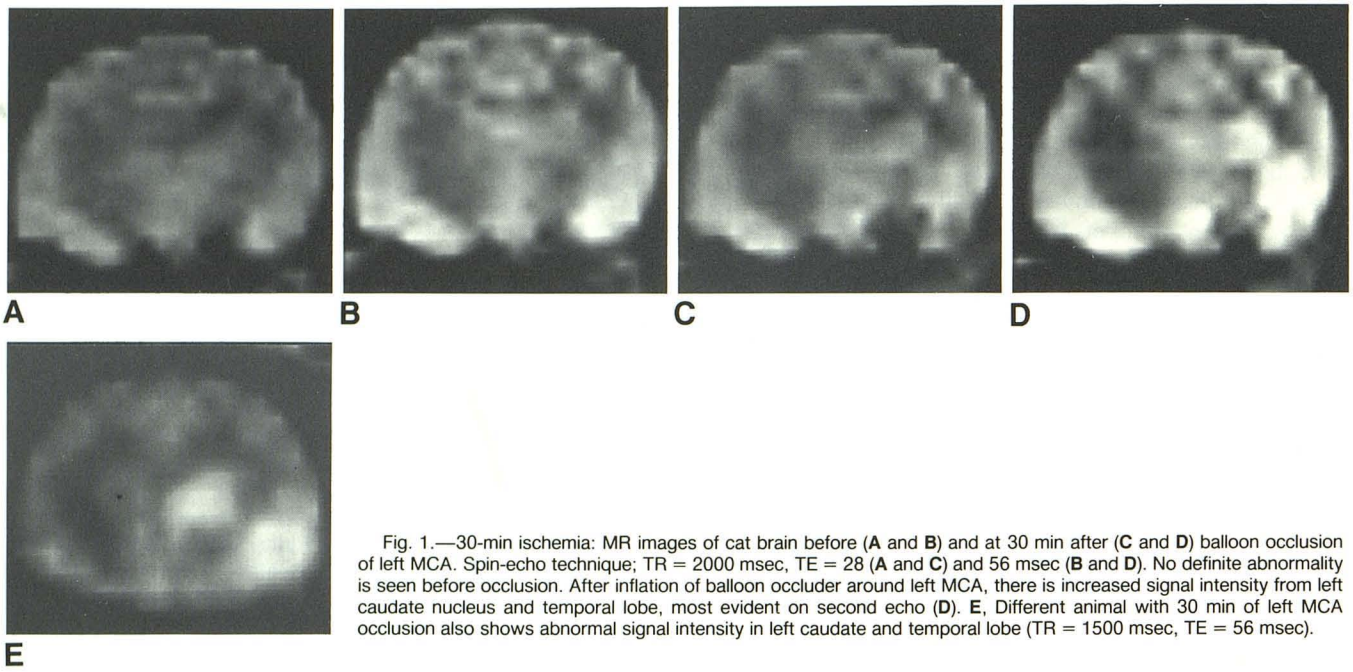

Fig. 1.—30-min ischemia: MR images of cat brain before (**A** and **B**) and at 30 min after (**C** and **D**) balloon occlusion of left MCA. Spin-echo technique; TR = 2000 msec, TE = 28 (**A** and **C**) and 56 msec (**B** and **D**). No definite abnormality is seen before occlusion. After inflation of balloon occluder around left MCA, there is increased signal intensity from left caudate nucleus and temporal lobe, most evident on second echo (**D**). **E**, Different animal with 30 min of left MCA occlusion also shows abnormal signal intensity in left caudate and temporal lobe (TR = 1500 msec, TE = 56 msec).

origin off the internal carotid artery and secured to the dura of the anterior clinoid with tissue adhesive. Temporary inflation of the balloon and consequent MCA occlusion (and its reversibility) was checked under microscopic visualization to verify occluder function. Gelfoam was used to seal the dura. The orbit was sealed by suturing the eyelid, with a tube connected to the occluder (and traveling through a subperiosteal tunnel in the orbit) protruding through the sealed eyelid.

Occluder inflation and deflation can be achieved by injection or aspiration of 0.5 ml of water into the connector tubing and balloon. After reversal of anesthesia, the animal can be tested neurologically to rule out a deficit associated with the surgery. Several hours before imaging, the occluder is again transiently tested in situ; the animal's sudden deficit with inflation and rapid resolution with deflation verify occluder function. For the purposes of imaging, anesthesia is again induced, and the cat is imaged before occluder inflation to rule out a preexisting, clinically silent lesion. The occluder is then inflated with the animal still in the magnet, and the animal is imaged. Deflation of the balloon allows subsequent changes of reperfusion, if present, to be imaged.

For this pilot study, the 13 cats were imaged at various intervals after ischemia. In five cats, images were obtained immediately after MCA occlusion to detect the earliest possible changes. One animal was imaged after 4 hr of MCA occlusion, three after 16–18 hr, one after 22 hr, and three more after 2, 3, and 7 days of occlusion, respectively; the 2-day animal was also imaged after 10 days. The animals were sacrificed and their brains removed as rapidly as possible after imaging.

The images were obtained with a clinical superconducting magnet operating at 0.35 T (Diasonics MT/S), the features of which have been described previously [15]. Initially, a 25-cm head coil was used for signal acquisition, but subsequently a smaller cylindrical coil 15 cm in diameter was constructed to improve the signal-to-noise ratio. The spin-echo technique was used with two sequences based on repetition times (TR) of 500 and 2000 msec (two echoes each) to allow calculation of T1 and T2 relaxation values. The slice thickness was 7 mm with a matrix of 128 × 128 or 256 × 256; the latter required twice the imaging time, thus was used only in the less acute insults. The long-TR sequence was used first to detect the abnormality, its duration being 17 min, and the midpoint (8.5 min) being taken as the point in time from occlusion to first data point, adding the tuning time and operator-computer interaction before initial RF excitation. The 500-msec sequence was used after a lesion was detected. An interactive cursor allowed definition of a region of interest for calculation of T1 and T2 values as previously described [2].

After imaging and animal sacrifice, the extracted brain was quickly sectioned in the coronal plane into 5-mm-thick blocks, which were examined grossly for signs of softening. These sections were then immersed in a 2% solution of 2,3,5 triphenyltetrazolium chloride (TTC). Because tissue infarcted before sacrifice does not stain with TTC, the lesion size can be grossly correlated with the MR image and contrasted to the normal hemisphere (infarcts less than 18 hr old show inconstant results with this stain). The tissue blocks were then stored in neutral-buffered formalin for later sectioning and staining with hematoxylin-eosin.

Results

The earliest changes of ischemia were observed on MR images at 30 min after balloon occlusion. Changes at 30 min were seen in two of the cats imaged through the first 3 hr of occlusion (fig. 1). In two other cats imaged through this time frame no changes were seen, while the fifth showed only equivocal alterations. The remaining eight cats with 4 or more hr of MCA occlusion all showed obvious evidence of ischemia on MR imaging (fig. 2). The regions involved always included the deep gray matter of the caudate nucleus; in 11 of 13 animals the globus pallidus and the temporal-parietal cortex supplied by the MCA were also included. All the lesions were

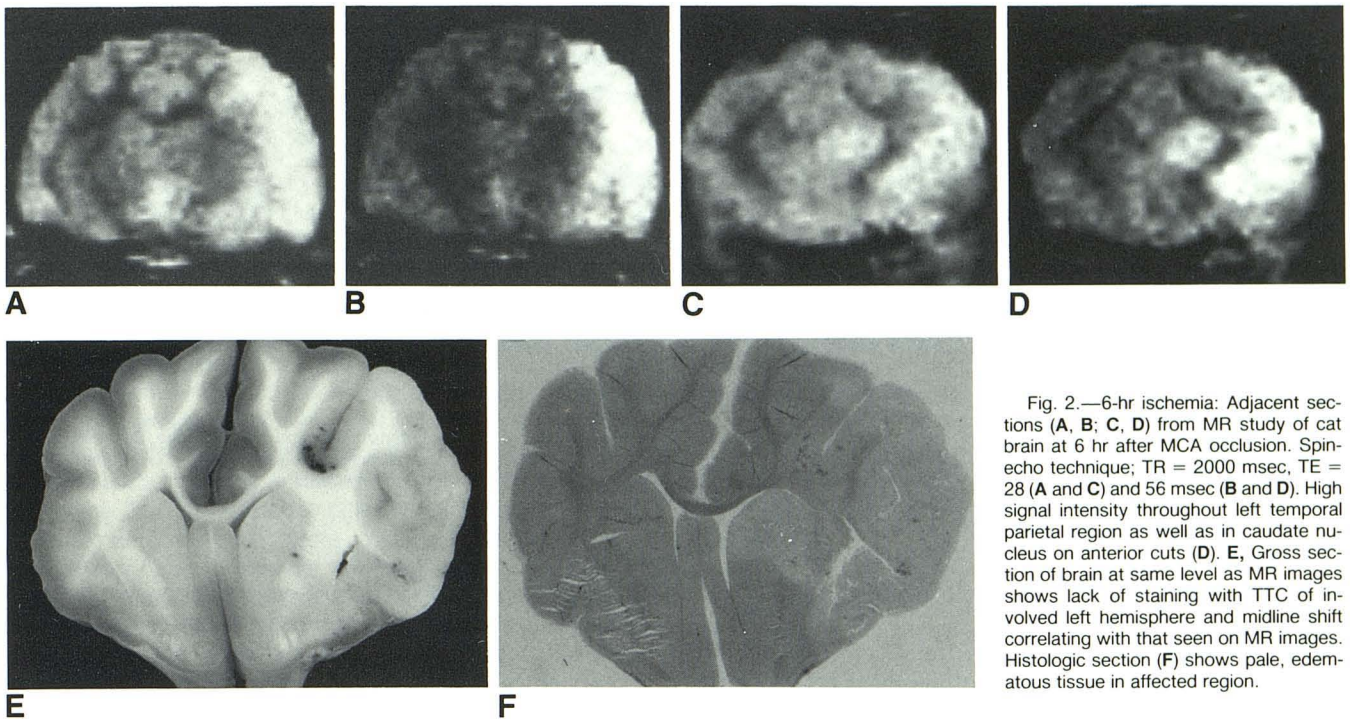

Fig. 2.—6-hr ischemia: Adjacent sections (**A**, **B**; **C**, **D**) from MR study of cat brain at 6 hr after MCA occlusion. Spin-echo technique; TR = 2000 msec, TE = 28 (**A** and **C**) and 56 msec (**B** and **D**). High signal intensity throughout left temporal parietal region as well as in caudate nucleus on anterior cuts (**D**). **E,** Gross section of brain at same level as MR images shows lack of staining with TTC of involved left hemisphere and midline shift correlating with that seen on MR images. Histologic section (**F**) shows pale, edematous tissue in affected region.

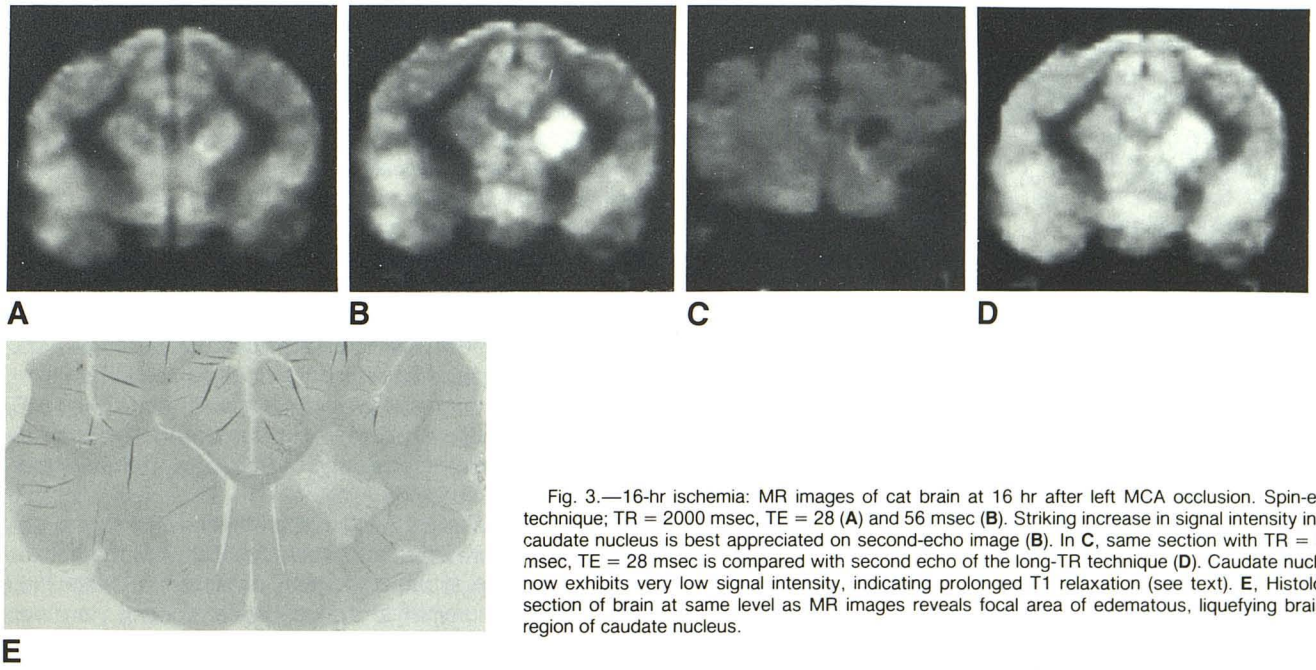

Fig. 3.—16-hr ischemia: MR images of cat brain at 16 hr after left MCA occlusion. Spin-echo technique; TR = 2000 msec, TE = 28 (**A**) and 56 msec (**B**). Striking increase in signal intensity in left caudate nucleus is best appreciated on second-echo image (**B**). In **C**, same section with TR = 500 msec, TE = 28 msec is compared with second echo of the long-TR technique (**D**). Caudate nucleus now exhibits very low signal intensity, indicating prolonged T1 relaxation (see text). **E**, Histologic section of brain at same level as MR images reveals focal area of edematous, liquefying brain in region of caudate nucleus.

evidenced on MR imaging by obvious increase in signal intensity on the T2-weighted (TR = 2000 msec) sequence, accentuated by the second echo. Little signal alteration was noted with the sequence using TR = 500 msec in seven cats, while three others showed low signal intensity in the involved gray matter. No obvious differences in the lesion distribution was noted between the 30-min insults and the less acute, 4-hr- to 7-day-old infarcts. The mass effect associated with the

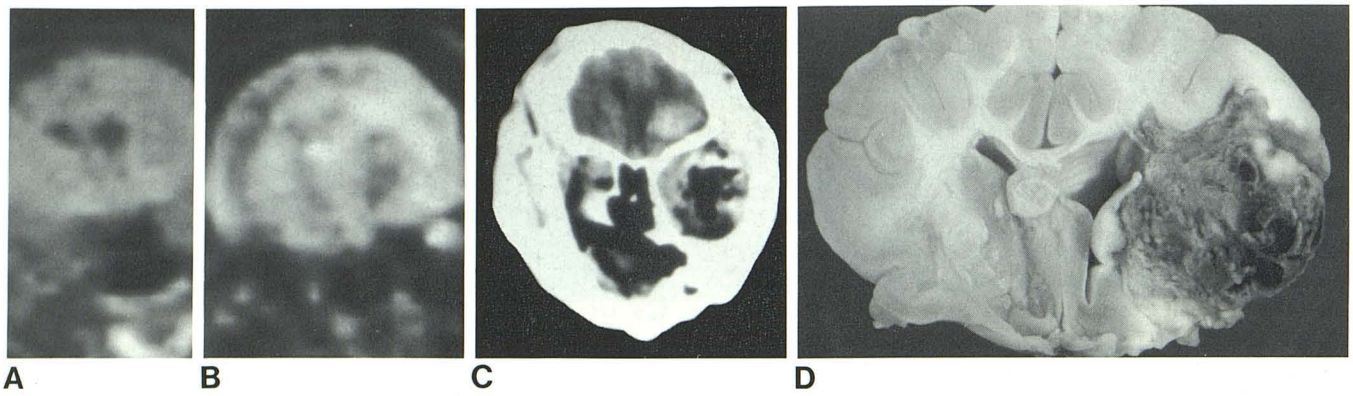

Fig. 4.—Hemorrhagic infarct: MR images (**A** and **B**) and CT scan (**C**) of cat brain at 10 days after permanent MCA occlusion. Spin-echo technique; TR = 500 (**A**) and 2000 msec (**B**), TE = 28 msec. MR images show midline shift; in **B**, brain edema is evidenced by abnormally high signal intensity in left parietal region. CT depicts focal high-density region in temporal lobe. **D**, gross section of brain of same animal, sacrificed immediately after MR and CT studies, shows organizing clot within an area of ischemic brain.

lesion did correlate with the age of the infarct, being greatest in the cat with the 3-day-old infarct.

Calculations of relaxation values were performed in nine of the 13 animals; minor movement of the head between the 500- and 2000-msec sequences made calculations in the others unreliable. The measurements of relaxation values showed a 12%–42% prolongation of T1 in the ischemic gray matter compared to the control gray-matter values (T1 = 726; range, 696–754 msec) in the opposite hemisphere. The T2 values of the lesions were 6%–24% longer as compared to normal (T2 = 53; range, 48–60 msec). Of interest, the greatest change in relaxation values (35%–42% increase) was found in the one 30-min lesion measured, and in two other infarcts less than 22 hr old (fig. 3). These three animals showed low signal intensity on the short TR sequences in the involved tissue. The paucity of data on old infarcts prevents any significant correlation between age of infarction and degree of T1, T2 value change.

Postmortem examination of the cat brain revealed obvious softening of the involved hemisphere in cases of ischemia of more than 16 hr duration, and lack of TTC staining (see Methods) on the sections correlating with the lesion size on MR imaging (fig. 2). TTC stain alteration and histologic changes were equivocal in the lesions with 30 min of MCA occlusion. Patchy areas of poorly defined softening were noted along with apparent neuronal shrinkage. The older lesions revealed more obvious looseness of the tissue and progressive pallor. The gray matter was more obviously involved than the white matter, with nuclear shrinkage, pyknosis, and coagulative necrosis, in that order, correlating with the lesion's chronicity.

Microhemorrhages were occasionally present. One cat, imaged at 2 days and again at 10 days after permanent MCA occlusion, showed a large organized focal hemorrhage on gross brain sectioning (fig. 4), corresponding to the lesion seen on MR imaging. Of interest, the animal underwent computed tomography (CT) (GE 9800) before sacrifice; the CT scan showed high density of the lesion which, on MR imaging, was not characterized as blood.

Only two animals in this initial project underwent reperfusion by deflation of the balloon occluder, one with a 30-min insult detected on MR imaging and one with a 4-hr insult. Subsequent MR images did not change, although the duration of reperfusion and imaging was kept short (up to 2 hr) for methodologic reasons.

Discussion

The results obtained in this study emphasize the unprecedented diagnostic sensitivity of MR imaging to the ischemic changes in brain tissue. Detection of ischemic insult to the brain within 30 min of production by a noninvasive, clinically pragmatic diagnostic tool is of major importance to both the researcher and the medical practitioner. Previous experiments in larger animals have suggested that MR imaging could detect ischemia of less than 2 hr duration [11], and studies in humans have shown the superiority of MR imaging over CT in depicting acute and chronic infarction [5, 6]. Our results support that superiority by demonstrating reliable detection by MR imaging of all infarcts of 4 hr duration or more. Also, our findings indicate the feasibility of using the established cat MCA-occlusion model for providing sufficiently good MR image quality of the small (4–5 cm diameter) feline brain to detect the subtleties of anatomy (including gray-/white-matter separation) and pathology.

Certainly, the likeliest explanation for the MR imaging abnormalities noted in the first hours of ischemia is the known 2%–3% increase in water content of ischemic tissue [16, 17]. Direct measurements of tissue water content were not performed post mortem in this first phase of our ongoing project, in part because of the methodologic problems of obtaining reliable measurements in the earliest stages of edema formation. Others have documented this early increase in water content of ischemic cat brain; the loss of high-energy phosphates leads to disturbed membrane homeostasis, intracellular edema, and subsequent leakage of water from the capillary space to the extracellular space, making up for the

earlier intracellular water shift [12]. The resultant increase in proton spin-density and lengthening of T2 relaxation in the affected tissue would produce the type of increased signal intensity seen with the long-TR spin-echo sequence in our animals. The relatively subtle changes on images obtained with the short-TR sequence reflect the known counterbalance of lengthened T1 relaxation versus the increased spin-density and T2 relaxation on signal intensity in edematous tissue [1].

The inability to detect ischemic changes in some of our animals in the first 3 hr of MCA occlusion may be related to the lack of adequate collateral supply. It is important to point out that when flow in an ischemic region is reduced to zero, the hydrostatic forces needed for progressive capillary leakage are absent and edema does not accumulate [12]. This may be one explanation for the lack of MR imaging changes in some animals studied in the first 3 hr. Given the unreliability of conventional histologic methods for verifying ischemic damage in the first 3 hr and the difficulties inherent in reliable measurements of tissue water content in the same time frame, other "gold standards" are needed for correlation of MR imaging changes (or their absence) in acute ischemia. Neurochemical analysis of fatty-acid accumulation, lactate levels, and tissue pH should prove more reliable and will be attempted in the future.

The ability of MR imaging to quantify the visualized ischemic insult on the basis of T1 and T2 relaxation values needs further investigation. The early extracellular edema that develops secondary to ischemia is relatively protein-free in comparison with the accumulation of protein-rich fluid subsequent to the eventual breakdown of the blood-brain barrier. Relatively pure water has longer T1 (and T2) relaxation values as compared to protein-rich water [18], possibly explaining the more marked prolongation of T1 in our very early ischemic lesions as compared to the older ones. This suggest that very early infarcts (before gross blood-brain barrier disruption) and very late infarcts (end-stage cystic lesions) may show longer T1 and T2 values than subacute, evolving infarcts that harbor protein-rich, vasogenic edema. Our model eventually will test this hypothesis. The availability of thin (2 mm) sections currently being installed into our MR instrument should provide more accurate data, less subject to partial-volume artifact.

The reversibility of an MCA ischemic insult with reperfusion has been recently reported in a baboon model [12]. This study suggested that regional perfusion must drop below 40% of normal for 30 min or more before edema formation begins. Reperfusion of the affected region exacerbates edema accumulation beyond these thresholds in proportion to the reperfusion. Such reperfusion within 30 min prevents an ischemic insult. Our data are insufficient to corroborate the study just mentioned, but the two cats that underwent reperfusion transiently after 30 min and 4 hr of ischemia, respectively, without alteration of the signal abnormality support the concept of irreversibility beyond a certain threshold. Of course, edema in and of itself need not correlate with severity of neurologic deficit or its permanence, and longer periods of reperfusion with subsequent MR imaging, behaviorial testing of the awake animal, and neurochemical/histologic correlation is needed.

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