Quantitative Proton Magnetic Resonance Imaging in Focal Cerebral Ischemia in Rat Brain

Helene Benveniste, MD; Gary P. Cofer, MS; Claude A. Piantadosi, MD; James N. Davis, MD; and G. Allan Johnson, PhD

Proton magnetic resonance (MR) imaging has been recommended as a diagnostic tool for the detection of focal cerebral ischemia. We compared microscopic MR images of rat brains after focal cerebral ischemia with evidence of histological damage found on corresponding silver-impregnated or cresyl violet-stained brain sections. Ten male Wistar rats were subjected to permanent unilateral occlusions of the right middle cerebral and common carotid arteries under halothane anesthesia. Twenty-four hours later the area of injury on MR images amounted to 26% of the total slice area, whereas only 9% of the total slice area was necrotic on histological sections from the same animals. The infarcted areas on tissue sections were surrounded by regions of selective neuronal injury in the cerebral cortex and occasionally in the hippocampus. The area of injury on MR images was larger than the combined areas of infarction and selective neuronal injury on histological sections. Areas of increased T2 values on MR images extended medially into noninfarcted striatum and laterally and dorsally into noninfarcted cortex. The lateral and dorsal areas on MR images frequently coincided with cortical areas in which considerable selective neuronal injury was present in the upper cortical layers. We hypothesize that the abnormal areas on MR images above histologically normal brain tissue represent the ischemic penumbra. If true, this is the first demonstration of the ischemic penumbra by MR imaging and may reflect our use of Wistar rats, a new image analysis technique, and ultra-high resolution MR imaging. (Stroke 1991;22:259-268)

Nuclear magnetic resonance (MR) imaging provides visualization of cerebral focal ischemia within a few hours after the interruption of cerebral blood flow (CBF) because the T1 (spin-lattice) and T2 (spin-spin) relaxation times become prolonged in ischemic tissue.1,2 Prolongation of relaxation times is often correlated with the presence of brain edema.3-6

When cerebral ischemia (e.g., occlusion of the middle cerebral artery [MCA]) results in an infarct, its area is defined histologically by the region in which all tissue elements are destroyed. There is an intermediate zone between the infarct and more normally perfused brain in which CBF is reduced to a level that interrupts neuronal function yet permits maintenance of membrane pumps and preservation of ion gradients.7-9 This area is referred to as the "ischemic penumbra."10 Because the CBF threshold leading to edema formation is roughly the same as CBF in the ischemic penumbra,11 it is reasonable to hypothesize that MR images might be abnormal in this region. If so, the total area of injury on an MR image should be larger than the infarcted area defined by histology. However, past studies of MR images in patients with acute stroke have demonstrated an excellent correlation with cryostatic sections,1 gross anatomic examination,12 and brain slices stained with 2,3,5-triphenyltetrazolium chloride (TTC).2-13 (TTC is a water-soluble salt that is reduced by mitochondrial enzymes in normal tissue to formazan, a deep red fat-soluble compound. The unique color provides a clear demarcation of damaged tissues, which do not stain red.) The surprisingly good correspondence between MR imaging and histological preparations could be due to several factors. The degree of edema in the ischemic penumbra may...
not be as pronounced as that in the infarct. The resolution of MR imaging may not be as good as that of histological sections. Finally, measurements of the area of injury on MR images and histological sections may not have sufficient precision.

The technology of MR imaging has continued to improve over the last several years. Spatial resolution has recently been extended into the microscopic domain. In view of these improvements, we studied the relation between areas of histological injury and changes on MR images after experimental ischemia using an approach different from that of previous studies. First, we chose a model of MCA occlusion in rats in which the infarct is generously surrounded by areas of selective neuronal injury. Second, we analyzed the extent of brain damage using a sensitive silver-impregnation stain in conjunction with a traditional cresyl violet stain. Third, MR imaging microscopy was performed with higher resolution and a higher signal-to-noise ratio than have been achieved in previous studies. Finally, areas of ischemic injury on MR images and histological sections were directly compared both manually and using an image analysis system.

Materials and Methods

The experiments were performed with Wistar rats because occlusion of the MCA in this particular strain is known to produce both infarction and selective neuronal injury. The 10 male Wistar rats (Charles River Laboratories, Inc., Wilmington, Mass.) used had free access to food and water until the day of surgery. The animals were divided into two groups. Rats in group I (n=7) were used for single-slice Carr-Purcell-Meiboom-Gill (CPMG) sequence and histological analysis 24 hours after occlusion of the MCA. Rats in group II (n=3) were used for a three-dimensional Fourier transform (3DFT) pulse sequence 24 hours after occlusion of the MCA.

On the day of surgery the rats (290-450 g) were anesthetized with 2.5-3% halothane in a 40% O₂ (balance N₂) gas mixture, intubated, and mechanically ventilated with a Harvard rodent ventilator (South Natick, Mass.). Anesthesia was maintained with 0.75-1% halothane, and the ventilator was adjusted to maintain normal PacO₂ and Paco₂. Because the MCA and the ipsilateral common carotid artery were occluded permanently, a polyethylene catheter was introduced into the right common carotid artery during surgery to allow the continuous monitoring of mean arterial blood pressure and the sampling of arterial blood for measurements of PaO₂, PaCO₂, and arterial pH (Model 813 blood gas/pH analyzer, Instrumentation Laboratory, Lexington, Mass.). The rat's temperature was monitored by a rectal thermometer and maintained at 37°C with an electrical heating blanket. A vertical 2-cm skin incision was made between the right eye and ear, and the parotid salivary gland was exposed. The vascular supply to the parotid salivary gland was occluded with two 3-0 silk sutures. The temporalis muscle was exposed and partly removed together with the zygoma so as to expose the inferotemporal fossa. Under saline irrigation, a craniectomy of approximately 2 mm diameter was made in front of the foramen ovale using a dental drill. The dura was opened with a sharp needle, and the exposed MCA was occluded with 10-0 sutures both proximal and distal to the lateral lenticulostriate branch(es) to increase the rate of infarction as described by Bederson et al. The soft tissue was put back into place, and the skin was sutured with 3-0 silk. The entire surgical procedure required 40 minutes. The rats in group II were exposed to the same general procedures as those described for group I, except that halothane was administered in a 3:1 N₂O:O₂ mixture during surgery and a different pulse sequence was used for MR imaging (see below).

All imaging was performed on a prototype 2.0-T CSI system (GE NMR Instruments, Fremont, Calif.) configured for MR imaging microscopy. Accurate measurement of T₂ is difficult, particularly in an imaging environment in which the presence of encoding gradients and the large, sometimes inhomogeneous, radiofrequency (RF) fields make even the most careful measurements suspect. Thus, in group I, T₂ was measured using a simple single-slice CPMG sequence, with careful attention to RF inhomogeneity. This particular sequence acquires eight echoes, with an echo time (TE) of 10-80 msec and a repetition time (TR) of 2.5 seconds (scanning time 43 minutes). Four excitations per phase encoding were employed to increase the signal-to-noise ratio, shielded gradient coils minimized residual eddy currents, a 6-cm low-pass “birdcage” RF coil provided good homogeneity, and a set of four CuSO₄·5H₂O solution standards (0.05, 0.025, 0.0125, and 0.00625 mM) with T₂ values ranging from 27 to 198 msec were included to assure precision. The four T₂ standards were first measured using a more accurate spectroscopy setting, with 64 echoes and much shorter RF pulses (data not shown). The field of view was adjusted to the smallest value that still included the rat and the T₂ standards (30-40 mm), yielding an inplane resolution of 117-156 µm. In group II, the 3DFT CPMG sequence that acquired four echoes (TE=20-80 msec, TR=2.5 seconds, scanning time 5 hours 42 minutes) at each of 16 contiguous levels was employed to provide volumetric coverage; no T₂ standards were included. In both groups the slice thickness was 2 mm. Calculated T₂ images were generated from the single-slice echo data by performing a two-parameter least-squares fit to the equation S=S₀e⁻ⁿ/TE/T₂.

In group 1, the calculated T₂ image was normalized by calculating a linear fit for the measured T₂ values of the four standards in the calculated images to their known values (measured spectroscopically). The majority of errors that might be encountered in an image are linear. Thus, should errors occur, scaling the entire image to the known T₂ values of the standards improves accuracy and precision when
comparing individual images. The drift of the measured T2 values from the known standard values was <20% over the course of the study.

Twenty-four hours after MCA occlusion the rats were anesthetized again with 3% halothane in a 40% O2 (balance N2) gas mixture, intubated, and mechanically ventilated. Anesthesia was maintained with 0.75–1.3% halothane during MR imaging. For ventilation, we used a small-animal modification of a ventilator described previously.20,21 Inspiration was initiated by the MR scanner on every other scan trigger pulse.21 This method of scan-synchronous ventilation permitted the MR imaging data to be acquired when there was no breathing motion.20,21 The rats were placed on an acrylic cradle designed to hold them stationary in the MR imaging apparatus for the duration of the study. While the animals were positioned in the magnet, their body temperatures were maintained by a continuous flow of heated air.

Immediately after completing MR imaging, the rats were deeply anesthetized with 80 mg/kg pentobarbital and perfused through the heart with 0.9% (wt:vol) saline for 1 minute followed by approximately 400 ml of 4% (wt:vol) paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. The brain was removed 1 hour later and placed in fixative at 4°C for 7 days and then in fresh fixative containing 30% (wt:vol) sucrose for 3 days. In group I, 40-μm-thick sections were cut with a freezing microtome and collected at 400-μm intervals starting 2.7 mm in front of and extending 3.3 mm behind the bregma.22 In group II, 40-μm-thick frozen sections were cut 2.2, 1.2, and 0.2 mm in front of and 3.1, 5.3, and 9.8 mm behind the bregma.22 Every second section in group I and all sections in group II were stained by the silver-impregnation procedure of Gallyas et al23 as modified by Crain et al.24 Briefly, the sections were washed with water, pretreated with alkaline ammonium nitrate, impregnated with 0.30–0.35% (wt:vol) silver nitrate in alkaline ammonium nitrate, treated with ethanolic sodium carbonate/ammonium nitrate solution, and finally developed in Nauta reducer. Adherent sections in group I were stained with cresyl violet.

Histological sections were matched with the MR images using the shape of the corpus callosum, striatum, and lateral ventricles. In our evaluation of brain tissue damage, we defined necrosis as regions in which all tissue elements were destroyed and selective neuronal injury as regions in which some neurons were injured while others appeared unharmed.

The MR images along with their corresponding silver-impregnated and cresyl violet-stained sections were analyzed for infarct size and hemispheric swelling, respectively, using an image analysis system (Image 1.26, Apple MacIntosh program). Data are expressed as mean±standard error of the mean; p<0.05 was considered significant. The significance of differences between groups was evaluated using the non-parametric Mann-Whitney U test, and paired data were analyzed using Wilcoxon’s paired rank sum test.
FIGURE 1. Top: Coronal section at level of caudate-putamen in rat 24 hours after occlusion of right middle cerebral artery and ipsilateral common carotid artery (x7.2, silver-impregnation stain). Infarcted area in dorsolateral striatum is black and easily distinguished from normal, noninfarcted tissue. Olfactory and frontoparietal (somatosensory area) cortex is partly infarcted. Bottom: Coronal section at level of dorsolateral striatum in rat 24 hours after occlusion of right middle cerebral artery and ipsilateral common carotid artery (x57.1, silver-impregnation stain). Necrotic, black tissue is sharply demarcated from normal, yellowish-brown brain tissue (arrow). Note that tissue architecture of necrotic striatum is preserved and corpus callosum (stars) is unharmed at this time.
FIGURE 2. Digitized images of (top) cresyl violet-stained and (bottom) silver-impregnated rat brain sections at level of caudate-putamen. Resolution of digitized images was 45×45 μm. Infarcted tissue, represented by pale and black area in top and bottom, respectively, was easily outlined and measured by image analysis program.

darkly stained neurons in the cresyl violet-stained sections. The middle cortical layers appeared normal (Figure 4, top). Occasionally, degenerating neurons were seen in columns perpendicular to the cortical surface. In two rats with large cortical infarcts selective neuronal injury in the upper and lower cortical layers was present several millimeters from the infarct rim (Figure 4, top). The measured areas of injured cortex included both infarcted brain tissue and tissue with selective neuronal injury and normal cortical tissue. These areas were quite variable, as demonstrated in Figure 3, bottom.

Selective neuronal injury was also present in the hippocampus ipsilateral to the infarct. In 70% of the rats, degenerated neurons (pyramidal cells) were demonstrated in the CA1 and CA3 regions and in the hilus of the dentate gyrus (Figure 4, bottom). The hippocampus contralateral to the infarct showed no evidence of neuronal damage.

In group 1, measurements of the area of the left and right hemispheres on cresyl violet-stained sections demonstrated that the right side was significantly larger than the left (50±1 mm² versus 48±1 mm², p=0.04). Hemispheric swelling was not detectable in the silver-impregnated sections, in which processing may have produced dehydration.

Infarct size evaluated by MR imaging measured by visually matching two images is subject to observer bias. We used an additional analysis procedure to eliminate the subjective bias when manually outlining a region of injury. This procedure was designed to not overestimate the region of involvement on the MR images. Histograms from T2-calculated images were obtained from corresponding areas in normal (left hemisphere) and infarcted (right hemisphere) regions (Figure 5). Prolonged T2 values in the right hemisphere are evident. The histogram of T2 values in the left hemisphere is narrow and centered around a T2 value smaller than that at the center of the right hemisphere histogram. We subtracted the distribution of the left-hemisphere T2 values from that of the right hemisphere to yield a composite curve. We then chose the “crossing point” in this composite curve to

FIGURE 3. Bar graphs. Top: Infarct size (necrotic area) for each rat as percentage of total slice area from silver-impregnated brain sections at level of caudate-putamen. Bottom: Area of injured cortex (i.e., infarcted areas, areas with selective neuronal injury, and normal cortical tissue) for each rat as percentage of total slice area.
FIGURE 4. Top: Somatosensory cortex of rat 24 hours after occlusion of right middle cerebral artery and ipsilateral common carotid artery (×60.9, silver-impregnation stain). Within cortex, prominent degeneration is confined to upper layers (arrows), while middle and lower cortical layers appear unharmed. Bottom: Rostral right hippocampal formation of rat 24 hours after occlusion of right middle cerebral artery and ipsilateral common carotid artery (×60.9, silver-impregnation stain). Somatodendritic argyrophilia is evident in areas CA1 (large arrowheads), CA3 (stars), and CA4 (small arrowheads). Seventy percent of all 10 rats had hippocampal damage ipsilateral to infarct.
set the T2 threshold for injured tissue. This threshold is a conservative estimate since the distribution of T2 values in the uninvolved tissue allowed more "normal" pixels to be subtracted from the distribution of values in the infarcted tissue. Infarct size in T2-calculated images was measured in all sections from all rats in group I and in corresponding MR imaging sections from the three rats in group II using both the manual outlining of regions of interest and the composite curve method described above. The infarcted area determined by manually outlining the bright area (Figure 6) was not significantly different from that defined by using the composite curve (28±4 mm$^2$ versus 29±3 mm$^2$, p=0.8). In group I, 44±2% of the right (infarcted) hemisphere had T2 values above the threshold defined by the composite curve compared with 11±5% of the left (noninfarcted) hemisphere. Areas with elevated T2 values in the noninfarcted hemisphere were usually confined to regions with ventricular fluid (Figure 6).

In all rats, the area of the right (infarcted) hemisphere was significantly greater than that of the left (56±3 mm$^2$ versus 52±3 mm$^2$, p=0.014). Total brain volume measured from the T2-calculated 3DFT CPMG images (16 brain slices) of the three rats in group II was 3.4, 3.5, and 2.7 cm$^3$, respectively. Infarct volume was 625, 673, and 450 mm$^3$ (i.e., 18%, 19%, and 17% of total brain volume), respectively.

Although swelling of the right hemisphere was apparent on MR images, areas of the left and right hemispheres measured on T2-calculated images did not differ from those measured on corresponding silver-impregnated and cresyl violet-stained brain sections (Table 2). To avoid possible overestimation of the injured areas on MR images due to swelling, areas of injury on MR images and brain sections were compared as percentages of the total slice area. As illustrated in Figure 7, top, the area of injury determined by MR imaging (both manually and by the composite curve method) as a percentage of the total slice area was larger than the area of necrosis measured on corresponding silver-impregnated sections (26.4±4.0% versus 9.4±1.9%, p=0.013) in all rats. Even when cortical areas of selective neuronal injury were included, the MR imaging outline of injury was still larger (Figure 7, bottom; 26.4±4.0% versus 18.4±2.2%, p=0.014).

**FIGURE 5.** Histograms from T2-calculated magnetic resonance imaging scan of rat brain 24 hours after occlusion of right middle cerebral artery and ipsilateral common carotid artery. Histograms were obtained from corresponding areas in normal (left hemisphere, solid line) and infarcted (right hemisphere, interrupted line) regions. Prolonged T2 values in right hemisphere are evident. Histogram of T2 values in left hemisphere is narrow and centered around T2 value smaller than that at center of right hemisphere histogram. Distribution of T2 values in normal hemisphere was subtracted from that of infarcted hemisphere, yielding composite histogram (dotted line). Crossing point (arrow) in composite curve was chosen and defined T2 threshold for injured tissue.

**FIGURE 6.** T2-calculated magnetic resonance imaging scan of rat brain 24 hours after occlusion of right middle cerebral artery and ipsilateral common carotid artery. Image was generated from four single-echo (echo time 20–80 msec) T2-weighted images obtained by three-dimensional Fourier transform Carr-Purcell-Meiboom-Gill sequence. In-plane resolution and slice thickness were 117×117 μm and 2 mm, respectively. Region of injury in right hemisphere is represented by prolonged T2 values and appears bright. T2 scale (in milliseconds) on right.
TABLE 2. Areas of Rat Brain Hemispheres Measured by Three Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Left (mm²)</th>
<th>Right (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic resonance imaging</td>
<td>10</td>
<td>52±2</td>
<td>56±3*</td>
</tr>
<tr>
<td>Silver-impregnation stain</td>
<td>10</td>
<td>52±2</td>
<td>52±2</td>
</tr>
<tr>
<td>Cresyl violet stain</td>
<td>7</td>
<td>48±1</td>
<td>50±1f</td>
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Results are mean±SEM.

*p<0.05 different from left hemispheric area using Wilcoxon's paired rank sum test.

fp<0.05 different from right hemispheric area on T2-calculated magnetic resonance images using Wilcoxon's paired rank sum test.

By means of computer analysis we were able to superimpose MR images and images from corresponding silver-impregnated sections. We could thus precisely determine the degree of overlap in the area of injury defined by MR imaging and that defined by histology (Figure 8). The overlapping areas on MR images extended medially into noninfarcted striatum and laterally and dorsally into noninfarcted cortex. Necrotic areas in the striatum and cortex were always contained within the borders of the MR imaging-defined area of injury. Brain areas with selective neuronal injury in the cortex were usually, but not consistently (Figure 8), found within the borders of the MR imaging-defined area of injury. The lateral partition of the overlapping MR imaging area coincided only with areas of extensive selective neuronal injury. When selective neuronal injury was very sparse, the T2 values were not prolonged. For example, in group I rats in which the right frontoparietal cortex was not infarcted, the T2 values measured within areas of selective neuronal injury were not significantly different from those measured in the corresponding left side (73±3 msec versus 75±4 msec, p=0.300). When the three rats in group II with infarcts in the right frontoparietal cortex were included, the T2 values of the right cortex were significantly larger than those of the left (82±6 msec versus 69±6 msec, p=0.043). For both groups, T2 values within the infarcted striatal areas were significantly larger than those within the contralateral noninfarcted areas (112±6 msec versus 71±3 msec, p=0.009). In the one rat in which the infarct was hemorrhagic, the corresponding T2-calculated image had a black center due to the very short T2 values in this area (T2 of <25 msec).

Discussion

We found that the area of cerebral injury defined by MR imaging 24 hours after MCA occlusion was much larger than the necrotic area defined by histology. Computer analysis revealed that areas on MR images with abnormally high T2 values extended medially into noninfarcted striatum and laterally and dorsally into noninfarcted cortex. The lateral and dorsal extensions always occurred in either nondamaged cortical areas or areas demonstrating massive selective neuronal injury.

It is well-known that prolongation of T2 is correlated with elevation of water content within ischemic brain tissue, and differences in the area of injury detected by the two techniques may therefore represent water that has migrated beyond the margins of the infarct. However, we prefer the hypothesis that noninfarcted areas with elevated T2 values reflect the ischemic penumbra. The rationale for this hypothesis is as follows. First, the overlapping areas often coincide with brain regions known to be hypoperfused 1 and 3 hours after MCA occlusion. Second, when local CBF is reduced sufficiently to allow edema to develop, the T1 and T2 relaxation times increase above normal. Finally, ischemic cortical edema...
SHR revealed sharp transition zones between infarcted areas and the more normally perfused neocortex, whereas CBF in the border between infarcted and noninfarcted areas in Wistar rats did not have the same dramatic transitions. Thus, after MCA occlusion the ischemic penumbra in SHR was not as large or as pronounced as that in Wistar rats, perhaps reflecting a more limited ability of the collateral vessels to dilate in SHR than in normotensive rats.

One difference between our study and previous ones is that we measured infarct size on T2-calculated images and not on T2-weighted images. However, the same measurements on T2-weighted images (TE = 70 and 80 msec, data not presented) yielded similar results. Our MR imaging technique differed in other ways from those of previous studies. Among other parameters, we used different magnetic field gradient strengths, pulse sequences, in-plane resolutions, slice thicknesses, and signal-to-noise ratios. For example, slice thicknesses in the former studies ranged from 2 to 10 mm and in-plane resolutions from 300x300 to 585x585 μm. We used a slice thickness of 2 mm and an in-plane resolution of 137x137 (varying from 117x117 to 156x156) μm. Thick slices and limited spatial resolution could obscure boundaries between normal and injured tissues.

If our hypothesis is correct and MR imaging may sometimes include the area of infarction and its surrounding ischemic penumbra, the observation has important clinical implications. It might explain the large areas of increased T2 values early after infarction that decrease when the same individual is studied later. At the least, these observations extend the usefulness of MR imaging for studies of experimental stroke since the effects of therapy on both the infarct and the surrounding penumbra could be determined.

Acknowledgment
We wish to thank Dr. Barbara Crain, MD, PhD, for valuable advice with the neuropathological evaluation.

References


KEY WORDS • brain edema • cerebral ischemia • magnetic resonance imaging • rats
Quantitative proton magnetic resonance imaging in focal cerebral ischemia in rat brain.
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doi: 10.1161/01.STR.22.2.259

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