T2- and Diffusion-Weighted Magnetic Resonance Imaging of a Focal Ischemic Lesion in Rat Brain

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Background and Purpose: We sought to evaluate the application of T2-weighted and diffusion-weighted magnetic resonance imaging techniques in the study of a focal ischemic lesion in the rat brain.

Methods: Unilateral cortical infarcts were induced using the photosensitive dye rose bengal and 560 nm light irradiation. Magnetic resonance images were recorded from a total of 11 rats at selected intervals from 1.5 hours to several days after induction of the lesion. Parallel experiments were performed in which Evans blue dye was injected into the lesioned animals either immediately after lesion induction (n=11) or 1 hour before the animals were killed (n=11). The second procedure was designed to show regions of blood–brain barrier permeability to plasma proteins at the time of sacrifice, whereas the first procedure showed the accumulation and subsequent dispersion of plasma protein following disruption of the blood–brain barrier.

Results: Regions of the cortex highlighted by the T2-weighted images corresponded well to the pattern of dye staining seen from the first procedure while the diffusion-weighted images showed visual correspondence with the staining pattern obtained using the second procedure.

Conclusions: These results illustrate the complementary use of T2-weighted and diffusion-weighted magnetic resonance imaging in discerning the pathophysiology of developing lesions.

KEY WORDS • brain edema • cerebral ischemia • magnetic resonance imaging • rats

Proton magnetic resonance (MR) imaging is ideally suited to the detection and investigation of ischemic brain infarcts because of the associated edematous changes. Although the pathological changes resulting from an ischemic insult are complex, it is the comparatively late stage of events following an insult, involving water accumulation, that is readily detected using conventional spin-echo imaging protocols. The changes seen in the MR image result from altered T1 (spin-lattice) and T2 (spin-spin) relaxation times, both of which become prolonged in edematous lesions. The edema is seen as regions of reduced signal intensity on T1-weighted images, whereas signal enhancement is observed in T2-weighted sequences; of these, the T2-weighted imaging protocol is thought to be the more sensitive to edematous changes. However, during the first few hours of an ischemic insult only a slight overall increase in total water occurs, and consequently T1- and T2-weighted images are relatively unaffected. Moseley et al4 invoked a new approach to obtain image contrast by the application of diffusion-weighted (DW) MR imaging. In a cat model of cerebral ischemia resulting from large-vessel occlusion they were able to detect ischemic regions in the cat brain with DW imaging before any changes were seen with conventional T2-weighted protocols. Although the exact reason for these observations remains obscure, DW imaging appears to be a useful technique in the study of cerebral pathologies both in humans5–7 and in animal models of disease.8–10

We report the application of both conventional T2-weighted and DW imaging protocols in the study of a photochemically induced ischemic lesion in the rat brain.11,12 Unlike models of severe ischemia produced by large-vessel occlusion, immediate alterations in blood–brain barrier (BBB) permeability and rapid development of brain edema are known to occur in this model of stroke.13,14 We relate the MR images obtained to the distribution of extravascular plasma albumin, visualized by an intravenous injection of Evans blue dye. Comparatively little surgery is required to produce the lesion, and recovery is rapid with no observable long-term deficits. The noninvasive nature of MR imaging enables serial studies to be carried out on a single animal, to follow the progression of the ischemic lesion.
Materials and Methods

We used adult male Sprague-Dawley rats (Harlan Olac Ltd., Bicester, UK) weighing 250–300 g. The rats were anesthetized with a mixture of isoflurane (1.8%), nitrous oxide (76%), and oxygen (22%) and were immobilized in a Kopf stereotaxic frame. Rectal temperature was recorded and maintained at 37.5±0.5°C with a homeothermic blanket. The head was shaved, and a longitudinal midline incision was made to expose the skull. The right side of the skull was cleared of muscle and connective tissue. The light guide was positioned close to the surface of the right side of the skull with the center of the 3-mm-diameter light aperture at coordinates, with respect to the bregma, antero-posterior 0.0 mm, lateral 3.0 mm. The light source used was a 250-W metal halide lamp (Zeiss Superlux, Oberkochen, FRG); the light was passed through a heat-absorbing filter, a 560-nm filter, and then along a fiber-optic light guide of 3 mm diameter. Rose bengal dye (Sigma, Poole, UK; 5 mg/ml in saline, filtered through a 0.22-μm filter, 0.30 ml/100 g rat weight) was injected either through a femoral vein catheter or directly into a tail vein over 45 seconds. Immediately after injection of the dye the light was switched on for 15 minutes. At the end of the illumination period, the head wound was sutured. The leg wound was also sutured after removal of the catheter and ligation of the vein. Lignocaine (Astra Pharmaceuticals Ltd., Kings Langley, UK) was applied to all wounds before inserting sutures.

The MR imaging experiments were performed on rats anesthetized with halothane (0.5–1.5%) and oxygen delivered through a nose cone and placed in a custom-built probe incorporating the radio frequency coils. Respiration rate and rectal temperature were monitored throughout the procedure, and the temperature was maintained at 37±0.5°C by passing warm water through a heat exchanger machined into the animal platform. Animals anesthetized for the duration of an imaging experiment (2–4 hours) recovered with no apparent difficulty and could be used for subsequent imaging studies. The imaging experiments were performed at 2.35 T (Oxford Instruments Ltd., Oxford, UK) or on a single-slice AM-100 spectrometer (Coventry, UK) in conjunction with a Picker Vista MR system (Wemblу, UK). Radio frequency pulses were delivered by a cylindrical transmitter coil, and signals were received with a 2.5-cm-diameter surface coil placed above the brain. The two coils were tuned to 100 MHz and were decoupled using the transmitter and receiver coils described by Styles et al. and Bendall et al., respectively. Coronal spin-echo images with a repetition time (TR) of 3,750 msec, an echo time (TE) of 140 msec, and two averages per phase-encoding step (SE 3750/140, NEX 2) and DW images (SE 3775/140, NEX 4) were obtained through the regions of interest (ROIs) with a slice thickness of 2 mm and a field of view of 50 mm. The DW spin-echo images were obtained as described in detail by King et al. The diffusion-sensitizing gradients (30 mT m⁻¹) were applied in the read direction (i.e., vertically up the image). T2 relaxation times were determined using a single-slice multiecho imaging procedure (TE of 30, 60, 90, and 120 msec; TR of 2,000 msec; NEX 4) and calculated from the least-squares fit to the semilog plot of normalized signal intensity versus delay time for the ROIs as defined below.

In the MR imaging experiments a total of 11 rats were included, five of which were imaged at each time, namely, the day of lesion induction (1.5–6 hours) and 1 day (24–30 hours), 2 days, 3 days, and 5 days following lesion induction. The data from these five rats were used for all subsequent statistical analyses and the T2 relaxation time determination. Signal intensity ratios, defined as the ratio of the mean signal intensity (noise subtracted) recorded from the ROI within the lesioned cortex to the mean signal intensity (noise subtracted) from the equivalent region in the contralateral hemisphere, were determined for the T2-weighted and DW images. Two ROIs were defined from the DW image recorded 1 day following lesion induction when the lesion appeared as two distinct regions in all animals examined. Region 1 is described as the core of the lesion and is defined as the volume of the cortex directly under the light source, centered 3 mm from the midline to a depth of 1.5 mm in the cortex. Region 2 forms the periphery of the lesioned volume and is bordered by the corpus callosum and the midline (as illustrated in Figure 3 insert). The ROI measurements were made from as many pixels as possible without extending into the adjacent region. All values are expressed as mean±SEM.

Unless otherwise stated, statistical analysis was performed via a repeated-measures analysis of variance. These calculations were carried out using the SAS statistical package (SAS Institute Inc., Cary, N.C.) via the REPEATED statement within the GLM procedure. Probabilities are derived from F ratios after making a degrees of freedom adjustment (Greenhouse-Geisser e) to compensate for the departure from sphericity. Subsequent univariate subeffect tests were restricted to two in number to control the overall error rate. Logarithmic transformations were applied to the data to improve the homogeneity of the variances.

There were two series of experiments (11 rats in each) in which Evans blue dye (Sigma, 10 mg/ml in saline, filtered through a 0.22-μm filter, 0.25 ml/100 g rat weight) was used. In one series, the dye was injected into the femoral vein immediately after induction of the lesion. The animals were allowed to recover from the anesthesia and killed with pentobarbitone sodium (RMB, Animal Health Ltd., Dagenham, UK) at the required times afterwards. The stained regions of the brain indicated the path of dispersion of the Evans blue/albumin complex after leakage through the disrupted BBB. A second series of dye experiments was performed at various times after induction of the cortical lesion to identify the regions of the brain in which the BBB remained leaky. Tail vein catheters were inserted, and dye was injected 1 hour before the rat was killed with pentobarbitone sodium. In both series, the brains were removed from the skulls immediately after death and photographed intact. The brains were then cut into 2-mm-thick coronal slices, and each slice was photographed to show the extent of the dye/albumin complex leakage.

Results

Progression of the lesion in one rat can be seen from the series of T2-weighted MR images recorded at 1.5
Figure 1. Coronal T2-weighted spin-echo images (repetition time 3,750 msec; echo time 140 msec; two averages per phase-encoding step) showing development of lesion over 5 days for one rat. a: Image recorded 1.5 hours following cortical irradiation. Area of high signal intensity seen above skull (open arrow) is thought to be due to inflammation associated with surgery. Lesioned volume can be seen as area of high signal intensity within right cortex (solid arrow). b: One day following lesion induction there is intense signal from lesioned volume together with hyperintense region in corpus callosum (large arrow) and, to a lesser extent, outer cortical lamina (small arrow). c: Image recorded 2 days after lesion induction. d: Image recorded 5 days after lesion induction. In c and d T2 changes are still detected in lesioned volume, although increased heterogeneity is seen.

Hours, 1 day, 2 days, and 5 days after lesion induction (Figure 1). The intense signal arching over the top of the brain is from the injured skin and connective tissue that had been separated from the surface of the skull. Rats imaged within a few hours after lesion induction all showed an increase in signal intensity in the volume of the cortex occupied by the lesion (Figure 1, a). The region of greatest signal intensity was within the core of the cortical lesion and spread, with diminishing intensity, laterally along the ipsilateral corpus callosum and, to a lesser extent, into the contralateral hemisphere over 24 hours (Figure 1, a and b). The T2 changes were most apparent 1 day after lesion induction, in both the lesioned cortex and the arc of high signal intensity within the corpus callosum and outer cortical lamina (Figure 1, b), with a gradual decline over the next few days. By day 5 (Figure 1, d), the T2 changes were still detectable but were less extensive and less pronounced than those of the preceding days. The signal intensity seen on the T2-weighted images correlated well with the T2 values presented in Table 1 (region 1, p<0.001; region 2, p<0.008; Spearman's rank-order correlation).

The DW images were routinely recorded after acquisition of the T2-weighted images. The DW images obtained on the day of lesion induction revealed an intense signal in the region of the lesion (Figure 2, a). It is interesting to see that in the DW images there is no signal associated with the injured skin overlying the skull. One day after induction of the lesion the DW images highlighted an intense halo around all but the pial surface of the lesioned volume (Figure 2, b). Unlike the T2-weighted images recorded at this time, the hyperintensity was restricted to this region and did not appear in association with the corpus callosum or the

<table>
<thead>
<tr>
<th>Time following lesion induction</th>
<th>Region 1 (msec)</th>
<th>Region 2 (msec)</th>
<th>Contralateral hemisphere (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5-6 hours</td>
<td>90±6</td>
<td>112±11</td>
<td>66±1</td>
</tr>
<tr>
<td>24-30 hours</td>
<td>118±17</td>
<td>128±4</td>
<td>64±3</td>
</tr>
<tr>
<td>2 days</td>
<td>84±2</td>
<td>118±6</td>
<td>65±2</td>
</tr>
<tr>
<td>3 days</td>
<td>79±2</td>
<td>116±9</td>
<td>65±1</td>
</tr>
<tr>
<td>5 days</td>
<td>68±2</td>
<td>97±2</td>
<td>64±2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=5.
FIGURE 2. Diffusion-weighted spin-echo images (repetition time 3,775 msec; echo time 140 msec; four averages per phase-encoding step) of rat brain collected immediately after acquisition of T2-weighted images shown in Figure 1. a: 2 hours, b: 1 day, c: 2 days, and d: 5 days following lesion induction. Signal intensity profiles (nonstandardized) are shown across lesioned volume as defined by straight line. Trigeminal nerves are highlighted (arrow in a).

outer cortical lamina. Approximately 24 hours later the halo had faded considerably (Figure 2, c), and it was not present in rats with 5-day-old lesions (Figure 2, d). The changes in DW image signal intensity with development of the lesion, recorded from the core (region 1) and the periphery (region 2), are shown in Figure 3; for comparison, the T2 data are also shown. Statistical analysis (two-way repeated-measures analysis of variance; imaging mode × time) indicates that there are significant overall changes with time (region 1, \( p < 0.0001 \); region 2, \( p < 0.002 \) [from the Greenhouse-Giesser-adjusted \( F \) ratio]) and that the time dependence differs between imaging modes (region 1, \( p < 0.025 \); region 2, \( p < 0.01 \)). Subsequent subeffect tests indicated that the differences between the DW and T2-weighted imaging modes performed the day of lesion induction were significant (region 1, \( p < 0.0007 \); region 2, \( p < 0.006 \)) and that the signal intensities seen in the T2-weighted images were significantly greater at 1 day than at 2 days following lesion induction (regions 1 and 2, \( p < 0.02 \)).

The pattern of staining produced when Evans blue was injected immediately after lesion induction is shown in Figure 4. The time course of dispersion of the stain (Figure 4) closely resembles the temporal changes seen in T2-weighted images (Figure 1). Quantitative comparison of the MR images and the Evans blue stain is problematic because different animals were used for each procedure and precise matching of slice position is difficult to achieve. Nevertheless, there is a convincing visual similarity between the development of T2-weighted imaging changes and dispersion of the dye from the center of the lesion at 1.5 hours (Figure 4, a) into the corpus callosum at 24 hours (Figure 4, b) and eventual removal from all but the core of the lesion at 5 days (Figure 4, d). The time course and distribution of the dye staining are consistent with extensive leakage of the dye/albumin complex from regions of BBB disruption within and around the lesion shortly after the lesion was produced. As the infarct develops, the dye/albumin complex is trapped in necrotic regions and is not readily accessible to phagocytes. From the periphery of the infarct, dye/albumin molecules move away from the lesion along the path of least resistance and are eventually removed by phagocytosis.

Blue-stained regions in the second series of animals, in which the dye was injected 1 hour before sacrifice (Figure 5), indicate that in the hour before death vessels in these regions were patent and the BBB was permeable to plasma proteins. Between 1 and 4 hours after lesion induction, most of the lesioned volume is accessible to the dye (Figure 5, a). Approximately 24 hours later the center of the lesioned volume is unstained and the dye appears as a halo surrounding the lesion (Figure 5, b). No tracking of the dye around the corpus callosum.
was seen. Two days after lesion induction both the intensity and the extent of staining had diminished (Figure 5, c), and by 5 days only a faint blue coloration confined to the rim of the infarct was apparent (Figure 5, d).

The pattern of dye staining seen as the lesion developed is remarkably similar to the regions of hyperintensity seen from the DW images obtained at comparable times (compare Figure 2 with Figure 5).

Discussion

Consistent with previous findings, our results show that edematous lesions are readily detected by T2-dependent spin-echo sequences. The hyperintense regions observed on the T2-weighted images correspond closely with the extravasation and dispersion of plasma protein tracer over the period studied (compare Figure 1 with Figure 4). Our results are in good agreement with previous reports showing that passive leakage of protein tracer occurs across the BBB almost immediately following the endothelial damage that results from cortical irradiation. T2 enhancement was greatest in both regions 1 day after cortical irradiation (Figure 1, b). Grome et al and Dietrich et al measured the quantity of water in a similar cortical lesion at different intervals after induction and found the greatest increase to occur between 4 and 24 hours following the insult.

The edematous changes in regions distant from the irradiated cortex were most marked on the day after the lesion was induced. Movement of plasma from the site of a lesion is known to occur along fiber tracts that offer the least resistance. This effect was clearly seen in the corpus callosum and, to a lesser extent, in the outer

Figure 3. Graph of signal intensity ratios from core (open symbols) and periphery (filled symbols) for both diffusion-weighted (circles) and T2-weighted (squares) imaging experiments, recorded over 5 days following lesion induction in rats. Values are mean ± SEM (n = 5). Insert: Line drawing of coronal section of rat brain close to bregma, illustrating regions chosen for intensity measurements taken from magnetic resonance images. Region 1 is equivalent to core of lesion; region 2 is defined as periphery of lesioned volume.

Figure 4. Photographs showing distribution of Evans blue dye injected immediately after lesion induction in rats. Brains were examined at (a) 1.5 hours, (b) 1 day, (c) 2 days, or (d) 5 days after induction of lesion.
cortical lamina (Figure 4, a and b). The regions of high signal intensity seen in the T2-weighted images were closely similar to those showing Evans blue staining when the dye was administered immediately after lesion induction.

T2-weighted MR imaging has been extensively used to study cerebral edema associated with an ischemic insult. The early events following an ischemic injury are not, however, readily detected by conventional imaging protocols. Recently it has been shown that, by making the imaging sequence sensitive to the diffusion properties of water, pathophysiological changes resulting from occlusion-induced ischemia can be seen before any enhancement is apparent on T2-weighted images. In fact, an ischemic region has been detected as early as 14 minutes after the onset of ischemia. In models of severe ischemia produced by large-vessel occlusion, early alterations in brain water are thought to be due to cytotoxic factors. In the rose bengal model of focal cerebral infarction, disruption of the BBB and consequent vasogenic edema occur almost immediately after the irradiation, and therefore the early stage of cytotoxic edema is not seen. Despite these differences, our results show that DW imaging can provide information concerning the nature of the lesion that is not obvious from T2-weighted protocols. The area of hyperintensity seen in the DW image within the core of the lesion (region 1) was rapidly lost over the first 24 hours (compare Figure 2, a with Figure 2, b); by this time the signal intensity ratio recorded from the core of the lesion was less than that from the equivalent region on the T2-weighted image (Figure 3) and remained so over the subsequent days. The difference between the two imaging modes during the later period (1–5 days after lesion induction) is significant (p<0.013). Since the DW images have the same T2 weighting, this signal attenuation is consistent with the diffusion of water being less impeded in this region of the brain. This may be a consequence of the developing necrosis. Such changes are not apparent in conventional T2-weighted images (compare Figure 1, b with Figure 2, b), which do not, therefore, distinguish these areas from the edematous but nonnecrotic periphery.

The pattern of signal hyperintensity in the DW images with development of the lesion shows a close correspondence to the pattern of Evans blue staining obtained when the dye was administered 1 hour before sacrifice (Figure 5). Therefore, DW imaging appears to highlight areas within the cortex in which the blood vessels are patent and the BBB is permeable to protein. Three recognized changes associated with increased permeability of the BBB in lesioned brain are 1) an increase of pinocytotic vesicles in endothelial cells, 2) hydration or thickening of the basement membrane, and 3) swelling of the pericapillary astroglial processes. Although we are at present unable to offer a precise description of the morphological alterations that account for the changes seen using DW imaging protocols, we have demonstrated that DW imaging gives information that is complementary to conventional T2-weighted imaging protocols. Identifying the underlying mechanisms responsible for these changes will yield a better understanding of the altered pathophysiology.

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