Serial Diffusion Tensor MRI After Transient and Permanent Cerebral Ischemia in Nonhuman Primates

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Background and Purpose—We measured the temporal evolution of the T2 and diffusion tensor imaging parameters after transient and permanent cerebral middle cerebral artery occlusion (MCAo) in macaques, and compared it to standard histological analysis at the study end point.

Methods—Stroke was created in adult male macaques by occluding a middle cerebral artery branch for 3 hours (transient MCAo, n=4 or permanent occlusion, n=3). Conventional MRI and diffusion tensor imaging scans were performed 0 (acute day), 1, 3, 7, 10, 17, and 30 days after MCAo. Animals were euthanized after the final scan and the brains removed for histological analysis.

Results—Apparent diffusion coefficient in the lesion was decreased acutely, fractional anisotropy was elevated, and T2 remained normal. Thereafter, apparent diffusion coefficient increased above normal, fractional anisotropy decreased to below normal, T2 increased to a maximum and then declined. Reperfusion at 3 hours accelerated these MRI changes. Only the fractional anisotropy value was significantly different between transient and permanent groups at 30 days. Final MRI-defined fractional lesion volumes were well correlated with corresponding histological lesion volumes. Permanent MCAO animals showed more severe histological damage than their transient MCAO counterparts, especially myelin damage and axonal swelling.

Conclusions—Overall, the MRI evolution of stroke in macaques was closer to what has been observed in humans than in rodent models. This work supports the use of serial MRI in stroke studies in nonhuman primates. (Stroke. 2007;38:138-145.)

Key Words: brain imaging  ■  diffusion tensor imaging  ■  diffusion-weighted imaging  ■  experimental  ■  focal ischemia  ■  interventional neuroradiology  ■  magnetic resonance  ■  MRI  ■  neuroradiology  ■  pathology ischemia  ■  stroke

A detailed understanding of the serial evolution of the brain’s MRI relaxation and diffusion parameters is clinically important for the diagnosis and treatment of stroke patients. For example, it can be used to help estimate the age of a lesion,4 to establish rational time windows for stroke treatment,2 or perhaps to provide alternative outcome measures or “surrogate end points.”3

Many studies have reported changes in the brain’s MRI parameters during and after stroke, both in humans1,4–8 and in rodents.9–13 The temporal evolution of the MR parameters can be roughly separated into 3 phases: (1) normal transverse relaxation time (T2), reduced apparent diffusion coefficient (ADC), slightly elevated fractional anisotropy (FA; hyperacutely); (2) elevated and increasing T2, low but increasing ADC, and low and decreasing FA (subacutely); (3) high but decreasing T2, elevated and increasing ADC, and low and decreasing FA (chronically). The exact timing of these events depends on the lesion’s underlying pathophysiological changes (including the effect of reperfusion11,12,14) but is generally much slower in humans than in rodents.

Because of the intrinsic differences in stroke evolution in humans compared with rats, the use of a more appropriate animal model is warranted for stroke investigations.15 Several groups have investigated stroke in nonhuman primates in order to more accurately model human stroke.16,17 Paralleling known differences in the evolution of stroke pathophysiology between primates and rodents,16 one might also expect that MRI measures of stroke evolution in higher primates (such as macaques) would more closely model what is observed in humans than in rodents. This hypothesis has not been verified. Previous studies have used MRI and magnetic resonance spectroscopy to evaluate acute and chronic ischemic injury in monkeys18–21; however, the detailed temporal progression of the MRI parameters, including T2 and diffusion, from the hyperacute to the chronic stroke phase has not previously been reported.

The objectives of our study were to measure the differential temporal evolution of the T2 and diffusion parameters after transient and permanent stroke in cynomologous ma-
Materials and Methods

Acute Stroke Model

Focal cerebral ischemia was created in 7 adult male macaques (Macacca fascicularis, 7.7±1.2 kg, 6 to 12 years old). All procedures were approved by our institution’s animal care committee. For all procedures, animals were anesthetized with Propofol (300 μg/kg per hour) and Remifentanil (0.1 μg/kg per hour) and mechanically ventilated with a 20% oxygen/air mixture, to maintain end tidal CO2 between 30 to 40 mm Hg. For transient ischemia, an MRI compatible micro-infusion catheter was advanced, under X-ray fluoroscopic guidance, into a branch of the middle cerebral artery and left there for 3 hours.22 Reperfusion was achieved in the transient middle cerebral artery occlusion (MCAo) animals, by removal of the catheter. Permanent MCAo was created by injection of a small volume of cyanoacrylate adhesive mixed with ethiodol.

MRI

Immediately after occlusion, animals were transferred to a 1.5-T MRI Scanner (GE Signa). Initial diffusion scans were acquired ~30 minutes after MCAo. Scanning continued up to 6 hours, after which animals were recovered and scanned again at 1, 3, 6, 10, 17 and 30 days. Dual-echo T2-weighted (T2-wt), FLAIR and diffusion/perfusion–weighted imaging MRI scans were acquired.22 Coronal image slices were prescribed perpendicular to a line connecting the splenum and genu of the corpus callosum.

Trace ADC, FA and T2 maps were generated from diffusion tensor imaging and T2-wt scans respectively. Lesion volumes and values of MR parameters were measured by two experienced researchers (Y.L., H.E.D.: using a semiautomatic region growing method) on a slice-by-slice basis. At the hyperacute stage, ADC maps were used to outline the lesion, whereas in all follow-up scans T2-wt and T2-wt FLAIR images were used.

Histological Analysis

At study end point, animals were euthanized (overdose of sodium pentobarbital) and their brains removed and immediately fixed in 10% formalin. A custom-designed matrix was used to correctly orient the brain, to allow cutting of 2.5-mm coronal blocks which closely matched the planes of the MRI slices. These tissues were processed, paraffin-embedded, sectioned at 6 μm, and stained with hematoxylin and eosin (H&E) or Luxol fast blue. Sections were processed, paraffin-embedded, sectioned at 6 μm, and stained with hematoxylin and eosin (H&E) or Luxol fast blue. Sections were closely matched the planes of the MRI slices. These tissues were processed, paraffin-embedded, sectioned at 6 μm, and stained with hematoxylin and eosin (H&E) or Luxol fast blue. Sections were

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>Permanent, Mean±SD</th>
<th>Transient, Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spongiosis and edema</td>
<td>2.0±1.0</td>
<td>1.5±0.6</td>
</tr>
<tr>
<td>Myelin degeneration</td>
<td>3.7±0.6</td>
<td>1.3±1.3</td>
</tr>
<tr>
<td>Axonal swelling (spheroids)</td>
<td>2.3±0.6</td>
<td>0</td>
</tr>
<tr>
<td>Liquefactive necrosis and cavitation</td>
<td>3.3±1.2</td>
<td>2.0±0.8</td>
</tr>
<tr>
<td>Macrophage infiltration (gitter cells)</td>
<td>3.0±1.0</td>
<td>1.8±1.7</td>
</tr>
<tr>
<td>Vascular hyperplasia</td>
<td>3.3±0.6</td>
<td>0.5±0.6</td>
</tr>
</tbody>
</table>

*P<0.05.

TABLE 1. Mean Histological Lesion Scores, on a Scale of 1 (least) to 4 (most severe)

Results

Ischemic strokes were successfully induced in all animals. The perfusion–weighted imaging scans confirmed occlusion and successful reperfusion (3-hour MCAo). One animal with permanent MCAo was euthanized after 17 days attributable to its poor neurological condition (hemiparetic, minimally responsive, sustained midline shift). The physiological parameters of both groups remained within the normal range (before and after reperfusion).

MCI Changes

Figure 1 shows MRI data from a transient and a permanent ischemia animal, respectively. In the (30 minute) FLAIR images of the transient MCAO animal, the hyperintensity seen in the sulci is likely the result of contrast agent leaking into the cerebrospinal fluid because of a leaky blood brain barrier or petechial hemorrhage. The evolution of T2, ADC and FA values after the stroke followed the pattern previously described,4 and is plotted in Figure 2. The mixed effects model showed a significant (P<0.05) effect of time*group on FA, and a weakly significant (P=0.09) effect on ADC, but only when considering data up to 17 days. The results of individual t tests on the FA data and on the ADC data up to 17 days are shown on the plots.

Figure 2(a) shows the changes in stroke lesion volumes against time. The volumes were normalized to the initial volume measured from the diffusion–weighted imaging acquired at 30 minutes after occlusion. Although the initial lesion volume in the 3-hour stroke group (1.16±0.736 mL) was smaller than in the permanent group (3.71±3.31 mL), the lesion volume evolution showed a similar pattern in both groups.

ADC Evolution

ADC (Figure 2(b)) was reduced in both groups immediately after MCAo. In the transient MCAo group ADC increased after reperfusion, whereas it continued to decrease in the permanent MCAo group. ADC pseudonormalized (ie, returned to an apparently normal value) at about 24 hours in the 3-hour MCAo group. In the permanent group, ADC reached its lowest value at 24 hours, then increased and pseudonormalized after about 10 days. The normalized ADC in the 3-hour group was significantly higher than that in the permanent group between 3.5 and 24 hours. ADC continued to increase in both groups after pseudonormalization but at a greater rate in the permanent group. In both groups, ADC values converged at day 30.

volume for each brain was calculated by dividing the sum of the lesion areas by the sum of the ipsilateral hemispheric areas. Similarly, fractional hemispheric lesion volume was measured on the matching 30-day MRI FLAIR images.
Mean lesion FA (Figure 2(c)) was slightly (but not significantly) elevated compared with the that of the contralateral control region, up to 3 hours after MCAo in both groups. FA dropped rapidly after reperfusion and reached its lowest value by about 6 hours. In the permanent occlusion group, FA decreased at a much slower rate. The FA difference between groups reached significance after reperfusion. FA increased at
the chronic stage in 3-hour group, whereas the FA remained low in the permanent occlusion group and was significantly different at 30 days.

T2 Evolution

T2 relaxation time (Figure 2(d)) of the ischemic lesion was not significantly different from that of the contralateral hemisphere during the first 6 hours after MCAo in the permanent group and before reperfusion in the transient group. Immediately after reperfusion, T2 increased in the 3-hour group but slowly became elevated in the permanent group during the acute period. In both groups T2 was highest between the second and third days, and declined thereafter.

Histopathology

Figure 3 shows a scatter plot of relative lesion volumes measured from MRI and histology for all animals. A linear regression analysis showed that there was a strongly significant correlation between MRI and histologically derived fractional hemispheric lesion volume ($r=0.99$, $P=0.01$). In the permanent occlusion group the slope of the regression was 0.82 (not significantly different from 1.0), whereas in the reperfusion group the slope was 1.88 (significantly $>1.0$, $P=0.045$). A GLM analysis indicated that the slopes were significantly different ($P=0.01$) between groups.

Ischemic damage was seen in both transient and permanent MCAo groups (summarized in Table 1). The transition between infarcted tissue and surviving tissue was very sharp and well-demarcated. Histological sections, shown in Figure 4, illustrate the more pronounced types of damage seen. Overall, more severe damage was seen in the permanent occlusion group; evidence of locally extensive (confluent) axonal swelling and vascular hyperplasia were only seen in this group, and the degree of myelin degeneration was significantly greater. Most cases, whether transient or permanent occlusion, had infiltration of foamy macrophages or gitter cells which phagocytize necrotic tissue debris, but the degree of macrophage accumulation was greater in the permanent occlusion cases. The degree of liquefactive necrosis and cavitation was visually more pronounced in the permanent occlusion cases; however, there was no significant difference between groups on the liquefactive necrosis score because the smallest permanent lesion did not demonstrate cavitation.

Although the mean lesion size in the permanent group was greater than in the 3-hour group, lesion size alone cannot account for the greater severity of histological damage seen after permanent occlusion. The 2 smaller permanent lesions were comparable in size to the mean 3-hour lesion size, yet both these permanent lesions demonstrated more severe damage overall than the reperfused lesions. In summary, the chronic lesion changes that contribute to alteration in tissue architecture and cellular composition include infarction, liquefactive necrosis with cavitation, neuronal loss, myelin degeneration, axonal swelling, macrophage accumulation, vascular proliferation, and edema.

Discussion

Evolution of ADC and T2

Reports on the timecourse of diffusion and T2 changes in human stroke lesions varied widely because of the natural variance in stroke etiology and the limited number of scanning timepoints (Table 2). Most rodent stroke diffusion MRI studies focused only on the acute stage although a few studies sparsely sampled chronic lesion evolution. Therefore, the data shown in Table 2 are our best estimates of the relevant parameters from the publications indicated therein.

In nonreperfused stroke, ADC reached a minimum at $\approx44$ hours after stroke onset in humans, at 8 to 24 hours in rats and at at $\approx24$ hours in our study (Figure 2(b)). ADC pseudonormalization varied widely in humans with a mean around 230 hours, compared with 48 to 72 hours in rats, and at 200 to 300 hours in macaques (more similar to the human data). Maximum T2 elevation in the lesion occurred after $\approx140$ hours in humans, compared with 24 to 48 hours in rats and 72 hours in our study (Figure 2(d)).

Serial MRI studies of reperfused stroke in humans and rodents involved a wide variety of occlusion durations. Nevertheless, it is known that the timecourse of diffusion and T2 changes in human reperfused stroke is accelerated compared with nonreperfused stroke, with ADC pseudonormalization at around 100 hours $^{14}$ and maximum T2 elevation at around 10 hours. MRI studies have measured the stroke lesion evolution in rats for several days after ischemic intervals ranging from 10 minutes to 2.5 hours, $^{11,12,23}$ and our best estimate is that after 60 minutes or more of ischemia, ADC pseudonormalization occurs at around 72 hours and maximum T2 elevation between 24 to 48 hours. A shorter occlusion duration leads to more rapid ADC pseudonormalization, whereas a longer (2.5-hour) occlusion leads to a more rapid T2 elevation. Again, the ADC changes in rats after reperfused stroke seem to be more rapid than in humans; however, the time scale of maximum T2 elevation is more comparable. After 3 hours of MCAo in macaques, both ADC pseudonormalization and maximum T2 elevation occurred at around 24 hours.

Evolution of Diffusion Anisotropy

A few studies in rats $^{24}$ and humans $^{7,8}$ suggested slightly increased diffusion anisotropy in the hyperacute phase of stroke. We also observed a slight hyperacute increase in FA
in both the permanent and transient MCAo groups. The decrease in diffusion anisotropy in the subacute and chronic stages of stroke\textsuperscript{6,8} is greater and more robust than the initial increase and is thought to arise from the loss of the tissues’ structural integrity. We also observed progressively decreased FA in both groups; however, in the transient ischemia animals, FA rebounded by day 1 and began to increase, reaching significance by day 30. FA was the only MRI parameter which was significantly different between the groups at 30 days.

**Comparison with Histology**
A few studies have compared MRI and histology in animal stroke models. A tissue signature analysis of multiparametric

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**Figure 4.** H&E (a,b,d,f,g,h) and Luxol fast blue (c, e) stained sections of transient (a, b) and permanent (c through h) MCAo lesions. Inset final MRI scans indicate the location of the microscopic fields. (a, 4×; b, 20×) spongiosis, edema within the neuropil (score 2), with surrounding gemistocytes; (c, 4×) large well-demarcated infarct with myelin loss (score 4); (d, 40×) axonal swelling/spheroids (score 3) and (e, 40×) associated myelin vesiculation (score 4); (f, 4×) cavitated lesion (score 4) with (g, 40×) foamy macrophage accumulation and phagocytosis of necrotic tissue (score 4); (h, 40×) vascular hyperplasia and perivascular edema (score 4).
MRI data after stroke in rats showed good correlation with the histological score. Li et al. showed that ADC recovery after transient stroke in rats does not necessarily indicate the lack of ischemic injury. In primates, however, little work has been done beyond comparison of MRI and histological lesion volumes. Marshall et al. found good correspondence between final MRI and histological lesion volumes after permanent MCAo in marmosets although at 3 weeks MRI underestimated histological lesion volumes, possibly attributable to macrophage infiltration. Two other studies compared hyperacute or chronic MRI and magnetic resonance spectroscopy measurements at a single timepoint to histopathological changes. To our knowledge, however, no previous study has attempted to relate the serial diffusion MRI changes in reperfused and nonreperfused stroke with quantitative histopathology in primates.

The histological findings in our macaque stroke model are consistent with previous primate studies. Our data also shows general agreement between MRI and histology. A permanent infarct was apparent on MRI after both reperfused and nonreperfused strokes along with histological evidence of necrotic infarcts in both cases. Combined with the fact that we did not observe hyperacute reversal of the diffusion-weighted imaging abnormality after reperfusion, this observation indicates that 3 hours of MCAo in our model is sufficient to cause a permanent infarct, as expected.

Various factors, including tissue shrinkage and distortion and loss of material from the lesion core during histological processing, may act to change the apparent lesion size on the histological sections. Nevertheless, the final fractional infarct volume on MRI was well correlated with the infarct volume measured on H&E sections. The apparent MRI overestimation of the histological infarct volume seen in some reperfused animals may be attributable to a degree of tissue shrinkage in the lesion during histological processing. In addition, diffuse edema surrounding the lesion is not apparent on the H&E sections, yet it may contribute to elevated tissue T2 and thus tend to increase the apparent MRI lesion extent after reperfused stroke.

Elevation of T2 in the MRI lesions generally indicates an increase in local tissue free water content: elevated final lesion T2 seen on both groups is consistent with spongiosis and edema (and possibly perivascular edema) observed on histology. While ADC is sensitive to local water content, it is also strongly affected by changes in cellular ultrastructure on the scale of 10s of microns, as well as local cell density. Hence, the persistently elevated ADC also seen in both groups is likely attributable to spongiosis and liquifactive necrosis with cavitation although the infiltration of macrophages may, to a degree, ameliorate these effects.

It is interesting that 2 of the histological parameters that were significantly increased after permanent MCAo, compared with reperfusion at 3 hours, were axonal swelling and myelin degeneration. It is believed that myelinated axons contribute much of the observed diffusion anisotropy in the white matter tracts in adult brain, and it is therefore

| TABLE 2. The Temporal Evolution of MRI Parameters From Previous Stroke Studies |
|-------------------------------|----------------|----------------|----------------|
| Investigator                  | T_ADCmin     | T_ADCnorm     | T_T2max       |
| Human nonreperfusion          |               |                |               |
| Marks4                        | 30            | 120            | >100          |
| Schlaug3                      | 30 (10–100)   | 241            | ...           |
| Copen2                        | 18            | 216            | ...           |
| Yang8                         | ...           | 280 (48–480)   | ...           |
| Zelaya8                       | 100           | 200            | ...           |
| Lansberg1                     | ...           | 264 (192–336)  | 144 (120–160) |
| Munoz Maniega32               | ...           | 288 (240–336)  | ...           |
| Human tPA treatment           |                |                |               |
| Marks4                        | ...           | 105            | 10            |
| Knight4                       | 6–8           | 48             | 24            |
| Rat permanent*                | 24†           | 72–168         | 48            |
| Rat reperfusion*              |                |                |               |
| Li1† (30 minutes)             | ...           | 24–48          | 48            |
| Neumann-Haefelin12 (30 min)   | ...           | 48–144         | 24            |
| Neumann-Haefelin12 (60 min)   | ...           | 48–144         | 24            |
| Sotak14 (60 min)              | 2             | 72             | 48            |
| Liu23 (60 min)                | ...           | 72             | 24            |
| Neumann-Haefelin12 (2.5 h)    | ...           | 72             | 5             |

Times are in units of hours. tPA indicates tissue plasminogen activator; T_ADCmin, time to the minimum ADC value; T_ADCnorm, time to ADC pseudonormalization; T_T2max, time to maximum T2 value.
*Time started at ischemia initiation; †first follow-up timepoint.
tempting to attribute the significantly lower 30-day FA value in the permanent occlusion group to the more severe axonal damage. The apparent increase in FA up to day 30 in the reperfusion group may result from a chronic reduction in edema (leading to T2 reduction), which decreases the isotropic free water component of tissue diffusion, allowing the underlying anisotropic component to dominate again.

Limitations and Future Work
Our study compared diffusion MRI and histopathology after stroke at a whole lesion level. Although this approach has been used by most previous imaging studies of stroke, heterogeneity within the lesion, combined with difficulties spatially coregistering histological slides with in vivo MRI data, makes an accurate comparison with histopathology challenging. Our ongoing studies are addressing the registration issue and will also involve a more quantitative (digital) analysis of immunohistochemical slides (of glial activation, for example). Measures of the regional evolution of ADC, T2 and FA will permit identification of the evolution and ultimate fate of tissues on a voxel level and define detailed MRI tissue signatures of histological damage.

Significant variability in lesion volume was observed after both transient and permanent occlusion in our model. This makes small differences in lesion size hard to detect between groups; however, it also reflects the variation in lesion size and location seen in human stroke. An advantage of the use of MRI is the ability to measure lesion volume early and use it as a normalization factor in subsequent measurements.

An additional potential limitation of this study was the use of anesthesia during stroke induction and MRI. Anesthesia may depress cerebral blood flow and function, and affect the hyperacute evolution of the infarct, but it may also suppress a stress-induced exacerbation of cerebral ischemia in the acute phase.

The use of anesthesia was unavoidable for our MRI scans; however, anesthetic doses were kept as low as possible, and the animals awoke rapidly at the end of the experiments.

Conclusions
The overall pattern of ADC, FA and T2 changes in our primate model of permanent MCAo more closely parallels that observed in humans rather than in rats. The analysis of the temporal evolution of the MRI data allowed us to distinguish early reperfusion from nonreperfusion in spite of the individual variation in stroke lesion volume and in spite of the lack of a significant difference in final relative infarct volumes between the 2 groups. Although both permanent and 3-hour reperfused MCAo yielded permanent stroke lesions, striking differences were seen in the temporal evolution on MRI (most pronounced between 1 and 6 days after stroke onset), and permanent MCAo lesions yielded a significantly lower FA value at the end point. Histological damage (especially in myelin and axonally related indices) was greater in the permanent MCAo group. We also found a good correlation between final fractional MRI lesion volumes and infarct volumes measured from histological sections.

By comparing relative changes in MR-derived metrics, we can control for the natural variation in lesion size and detect changes in the strokes’ evolution, for example, attributable to a putative acute stroke therapy, with smaller group sizes. Our results thus support the use of serial MRI (including diffusion tensor imaging) in studies of cerebral ischemia and in the evaluation of novel stroke therapies using nonhuman primates.

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Disclosures
None.

References


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