Published in final edited form as:

Brain Res. 2010 November 11; 1360: 168–176. doi:10.1016/j.brainres.2010.08.098.

Chronic Brain Tissue Remodeling after Stroke in Rat: A One Year Multiparametric Magnetic Resonance Imaging Study

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Abstract

Rats subjected to 2 hours of transient middle cerebral artery occlusion were studied temporally over 1 year by magnetic resonance imaging (MRI) and behavioral testing. Multiparameter MRI measures of T_2 , T_1 , T_1 in the presence of off-resonance saturation of the bound proton signal (T_{1sat}), apparent diffusion coefficient (ADC) and susceptibility-weighted imaging (SWI) were obtained at 1 day, 1, 2, 3 and 4 weeks, and 3, 6, 9 and 12 months post-ischemia. Regions of interest included: ischemic core (damaged both at 1 day and later); new lesion (normal at 1 day, but damaged later); and recovery (damaged at 1 day, but normal later) areas. Hematoxylin and eosin, Prussian blue and ED-1, a monoclonal antibody murine macrophage marker, stainings were performed for histological assessment. Core area T_2 and ADC values increased until ~6 months, and T_1 and T_{1sat} until ~12 months. New lesion area MRI parameter values increased until ~6 months (T_2 , T_1 and ADC), or ~1 year (T_{1sat}). Lesion area was largest at 1 day (mean $\pm$ SD: 37.0 $\pm$ 13.7 mm²) and smallest at 1 year (18.1 $\pm$ 10.5 mm²). Recovery area was largest at 3 weeks (8.9 $\pm$ 3.8 mm²) and smallest at 1 year (6.4 $\pm$ 3.3 mm²). The ipsilateral/contralateral ventricle area ratio was 0.7 $\pm$ 0.2 at 1 day and increased significantly at 1 year (2.4 $\pm$ 0.7). Iron-laden macrophages, histologically confirmed at 1 year, were detected in the lesion borders by SWI at 3, 6, 9 and 12 months. Our data indicate that MRI detectable changes of ischemia-damaged brain tissue continue for at least 1 year post-ischemia.

Keywords

apparent diffusion coefficient; macrophages; middle cerebral artery occlusion; T_1 ; T_{1sat} ; T_2

1. Introduction

Magnetic resonance imaging (MRI) is well suited for long-term studies of experimental stroke. A detailed knowledge of the temporal evolution of various MRI parameters in

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Disclosure/Conflict of Interest: None

ischemia damaged brain tissue at both acute and chronic time points may provide useful information about the long-term time course of brain tissue injury and repair (Knight et al., 1994; Lansberg et al., 2001; Liu et al., 2007; Weber et al., 2006). Presently such temporal MRI investigations of post stroke tissue injury and recovery in rat brain have only been performed for a duration of months. To better understand evolution of tissue injury and repair processes, and the impact of therapeutic interventions, it is important to investigate the temporal profiles of such parameters for longer time periods. We postulate that ongoing changes may continue for up to 1 year after stroke.

There have been several longitudinal studies of animal stroke models reported using MRI parameters (Weber et al., 2006). One such study reported the accumulation of iron-laden macrophages on high-resolution 3D T_2^* -weighted MRI 2 – 10 weeks after stroke (Weber et al., 2005). Another showed that the temporal profile of T_1 and T_2 relaxation time changes over a 10 week time period could be used to discriminate lesion development into selective neuronal death or pannecrosis (Wegener et al., 2006). To the best of our knowledge, the longest period of post-stroke follow-up investigation in rats was 1 year after stroke (Modo et al., 2009; Shen et al., 2007). Shen et al. did not include MRI whereas Modo et al. included serial, T_2 -weighted MRI only over the period for the assessment of MRI contrast agent-labeled neural stem cell transplants. This study tests the hypothesis that changes in ischemia damaged brain measured by multiparametric MRI continue at least for one year after middle cerebral artery occlusion (MCAO). Effective MR parameters for long term assessment of ischemic brain injury and recovery were determined and documented for the temporal evolution until one year after MCAO. Neurobehavioral testing and histology studies were performed to generate complementary data to support the multiparametric temporal MRI assessment.

2. Results

2.1. Neurobehavioral tests

Descriptive statistics for modified neurological severity score (mNSS) and adhesive-removal test (ART) results are presented in Table 1. For both tests, higher scores indicate increased severity. The baseline mNSS score was 0 (normal function) and ART value was less than 10 s before stroke. The highest scores for both mNSS and ART were found 1 day post-stroke (mean $\pm$ standard deviation [SD]: 10.50 ± 1.38 and 120 ± 0 , respectively). The scores for all animals improved at later time points, with the best mNSS and ART scores obtained at 1 year (5.00 ± 0.89 and 46.17 ± 26.38 , respectively). The values for all later time points improved significantly as compared to 1 day. Mean ART value at 2 months was greater than that at 4 weeks, however, this was due to the use of smaller tabs from 2 months onwards to increase the sensitivity of the test.

2.2. MRI measurements

Fig. 1 shows T_2 , T_1 , T_1 in the presence of off-resonance saturation of the bound proton signal (T_{1sat}) and apparent diffusion coefficient (ADC) maps of the central coronal slice from a representative animal brain for all MRI time points studied. The maps visually demonstrate changes in the area and corresponding MRI parameter values of ischemia damaged tissue, and the continuity of ipsilateral ventricle expansion across the study time points.

The mean values ($\pm$ SD) of the core and recovery areas, or the core and new lesion areas, defined as the total lesion, and corresponding contralateral hemisphere regions of interest (ROIs) are shown in Table 2. At all time points, all MRI measurements in the recovery region differed significantly ($p \leq 0.05$) from those in the core region. These data are plotted in

Fig. 2. Core and recovery regions; and their corresponding contralateral regions were considered for the statistical analyses. The ratio of ipsilateral to contralateral (I/C) measures was higher in the core region as compared to the recovery region for all MRI measures from 1 week onwards. All MRI parameters showed large changes in the ischemia damaged area at early time points which tended to stabilize at later times whereas those of corresponding ROIs in the normal contralateral hemisphere remained almost constant throughout the study, indicating that the ongoing changes occurred in the damaged hemisphere only.

In four animals, areas of new lesion were identified by MRI. New lesion areas may be due to ongoing cell death or changes in the geometry of the injured brain. MRI parameter values (mean $\pm$ SD) for these regions are plotted in Fig. 2. The new lesion mean MR parameter values (T_2 , T_1 and ADC) increased significantly until ~6 months, or ~1 year (T_{1sat}) whereas for core region, T_2 and ADC had the highest mean values at ~6 months, and for T_1 and T_{1sat} , the highest mean values were at ~1 year post-ischemia. The variability in the mean values of both core and new lesion regions were generally larger at later time points. The MR values in the recovery region tended to become equal to those of the corresponding contralateral ROIs at later time points.

2.2.1. T_2 measurement—In the core, T_2 values were slightly higher than the total lesion mean and were significantly elevated at 1 day (92 ± 14 ms) and 1 week (83 ± 12 ms) post-ischemia relative to the corresponding contralateral regions (56 ± 5 ms and 54 ± 3 ms, respectively) (Fig. 2B). These data show an increasing trend in T_2 values from 1 week to 6 months (232 ± 111 ms) which then appeared to stabilize until 1 year (221 ± 110 ms). Values in the recovery region were 86 ± 13 ms at 1 day post-ischemia, then became almost constant and equal to those of the corresponding contralateral normal tissue region at all other time points. The mean T_2 values for the new lesion areas increased from 63 ± 6 ms at 1 day to a maximum of 166 ± 80 ms at 6 months (Fig. 2B).

2.2.2. T_1 measurements—Mean T_1 values for the core, recovery and new lesion areas, and the corresponding contralateral regions are shown in Fig. 2C for times from 1 day to 1 year. The mean T_1 values for the core decreased slightly from 1 day (1946 ± 293 ms) to 1 week (1646 ± 157 ms), and then increased from 1 week to 1 year (2513 ± 574 ms). The T_1 value of the recovery region was 1810 ± 239 ms at 1 day and then became almost constant at all other time points. The mean T_1 value of new lesion was highest at 6 months (2055 ± 375 ms).

2.2.3. T_{1sat} measurements—The T_{1sat} values for the core region at 1 day and 1 week post-ischemia were 822 ± 86 ms and 762 ± 144 ms, respectively (Fig. 2D). The core region values at later times, increased continuously until 3 months (1764 ± 725 ms), leveled off between 6 and 9 months, and then increased at 1 year (1975 ± 747 ms). In the recovery region, T_{1sat} values were elevated compared to the corresponding contralateral regions, but did not vary significantly across time. In the new lesion areas, the mean T_{1sat} values increased continuously from 619 ± 86 ms at 1 week to a maximum of 1364 ± 381 ms at 1 year.

2.2.4. Apparent diffusion coefficient (ADC) measurements—Mean ADC values for the core ($[5.84 \pm 0.74] \times 10^{-4}$ mm²/s) were significantly lower than the corresponding contralateral normal tissue regions ($[7.30 \pm 0.32] \times 10^{-4}$ mm²/s) 1 day after MCAO. Core values increased from 1 day to 6 months, and then showed little subsequent change to 1 year (Fig. 2E). At 1 day, the ADC values of the recovery region ($[5.83 \pm 0.86] \times 10^{-4}$ mm²/s) were significantly lower than the corresponding contralateral normal tissue regions ($[7.14 \pm 0.38] \times 10^{-4}$ mm²/s). Recovery region ADC values then became slightly elevated at later time points to $(8.13 \pm 0.85) \times 10^{-4}$ mm²/s at 1 year. The mean ADC values for new lesion increased

from $(7.15 \pm 1.09) \times 10^{-4} \text{ mm}^2/\text{s}$ at 1 day to a maximum of $(14.99 \pm 4.94) \times 10^{-4} \text{ mm}^2/\text{s}$ at 6 months, again showing little further change out to 1 year.

2.2.5. Volume and area measurements—The maximum area of ischemic damaged tissue ($37.0 \pm 13.7 \text{ mm}^2$) occurred 1 day postischemia using T_2 maps (Fig. 3). It decreased at all later time points reaching a minimum ($18.1 \pm 10.5 \text{ mm}^2$) at 1 year. For the recovery region, the mean area was largest at 3 weeks and reached a minimum value at 1 year, but there was no significant difference in the values out to 1 year post-ischemia. The ratio of ipsi/contra (I/C) lateral ventricles areas increased steadily from 1 day (0.7 ± 0.2) to 1 year (2.4 ± 0.7), at a faster rate for early time points and slower for later time points, suggesting continuous tissue loss to the ipsilateral lateral ventricles (LVs) not only for a short period as observed previously but also for later times at least for one year after stroke (Fig. 3). The lesion volume from hematoxylin and eosin (H&E) histological sections taken 1 year after stroke was $27.6 \pm 12.8 \%$ of the contralateral hemisphere.

2.3. Histology and susceptibility weighted imaging (SWI) results

Strongly hypointense regions were frequently observed near the lesion boundary on SWI sections acquired at various times out to 1 year post-ischemia (Fig. 4A) leading us to speculate that these regions may contain iron-containing cells or macrophages. Macrophages were observed by ED-1 immunohistochemistry (brown colored spots in Figure 4B and 4C) performed 1 year after stroke, with some containing iron as indicated by the colocalization with the blue iron-positive Prussian blue stained regions (arrow in Fig. 4C).

3. Discussion

The primary findings were that ongoing changes in brain tissues continued out to 1 year post-stroke. Continuous tissue loss in the ipsilateral hemisphere occurred during this period as indicated by the significant expansion of the ipsilateral ventricle after stroke, not only for a few months as previously observed, but also for later times out to at least 1 year. The presence of iron-laden macrophages in the ischemic boundary at 1 year post-stroke suggests an ongoing inflammatory process. Because of the small sample size used in the study, the data should be interpreted carefully. These data showed that ischemic brain continues to change over a 1 year period after stroke, and MRI measures of T_2 , T_1 , $T_{1\text{sat}}$ and ADC can be used to monitor these changes. The severity of behavioral deficits was high in all animals at the acute stage, but significantly improved over time.

In the present study, T_2 was found to be the most reliable MRI parameter to differentiate infarcted tissue from normal tissue. The T_2 -weighted MRI contrast in brain depends primarily on tissue water content and to a lesser degree on the interaction of free water with tissue macromolecules – proteins and other large and structurally fixed molecules (Ewing et al., 1999). Mean T_2 value in the lesion core was lowest at 1 week after MCAO, increased 1 week to 6 months, and then appeared to stabilize until 1 year. Similarly, mean T_1 values for the core region were initially elevated, lowest at 1 week, increased continuously to 6 months and thereafter appeared to level off. The small decrease of T_1 values at 1 week compared to the value at 1 day may be associated with a decline in tissue water content due to reduction of edema (Ewing et al., 1999) and/or an increase in the concentration of paramagnetic compounds (Gowland and Stevenson, 2003) in the tissue due to vascular degradation. Mean $T_{1\text{sat}}$ values in the core regions at 1 day and 1 week after MCAO were almost the same and were significantly higher than the corresponding contralateral regions (Fig. 2D), suggesting increased BBB disruption (Nagaraja et al., 2008). At later times, these phenomena progressed further in the core region until 6 months. In animals, ADC values in ischemia damaged tissue pseudonormalize at approximately 2 days and become elevated thereafter

(Schaefer et al., 2000). In this study, the average ipsi/contra ADC ratio was 0.83 ± 0.08 at 1 day, 1.19 ± 0.18 at 1 week, indicating elevated ADC values of lesion, and then leveled off at 6 months (2.54 ± 0.69) and 1 year (2.54 ± 0.75). The absolute ADC values in the lesion were $(1.77 \pm 0.46) \times 10^{-3} \text{ mm}^2/\text{s}$ at 6 months. These elevated ADC values were much higher than normal grey matter and white matter (Thomas et al., 2000), but still much smaller than that of 'free' water at 37°C ($3.0 \times 10^{-3} \text{ mm}^2/\text{s}$) (Le Bihan, 2003) or cerebrospinal fluid, suggesting less restricted movement of water molecules in the tissue.

The mean recovery area was largest at 3 weeks and reached a minimum value at 1 year, with no significant difference in the values. The mean values for all MRI measures for the recovery regions were slightly higher than the corresponding contralateral values after 1 week except for T_2 values. Hence these tissues tended to resolve, becoming normal as before MCAO. The T_2 , T_1 , $T_{1\text{sat}}$ and ADC values for new lesion were higher than the recovery regions, yet lower than core values at later time points as expected since these new lesion areas lie in the outer portion of the ischemia damaged lesion. Accordingly, these areas may have less water accumulation and/or a lesser degree of proteolysis of tissue than the core region.

The ipsilateral LV area was usually compressed and smallest at 1 day post ischemia due to brain edema. At later times, the size of the ipsilateral LV continuously increased relative to that in the contralateral hemisphere with cerebrospinal fluid appearing to replace some of the ischemia damaged brain tissue. Significant ipsilateral ventricular enlargement was evident even after a few months.

In a somewhat unexpected observation, dark regions were often noted at the borders of the infarcted tissue of the SWI sections at time points from roughly 1 month to 1 year. Weber et al. (Weber et al., 2005) reported the accumulation of iron-laden macrophages on histological sections, using Prussian blue (for iron) staining and immunohistochemistry for ED-1 positive macrophages, which corresponded well with hypointense signals detected at 10 weeks post-stroke on 3D T_2^* MRI. This led us to investigate whether iron containing macrophages were still present at 1 year. As speculated, macrophages (with some containing iron) were found on Prussian blue and ED-1 stained sections. These observations support the findings of Weber et al. and provide incentive for further study of the role of macrophages near the edges of the stroke damaged lesion at late time points. These also suggest an ongoing inflammatory response and phagotization of tissue out to 1 year post-stroke.

In summary, the findings of this study suggest ongoing changes in stroke damaged rat brain tissues can persist for at least one year. To the best of our knowledge, this is the first study to document the post-stroke temporal evolution of multiparametric MRI measures of T_2 , T_1 , $T_{1\text{sat}}$, and ADC values in aged animals out to 1 year. Although a small sample size was used in study, these data suggest that MRI can provide useful information about the long term time course of brain injury and repair.

4. Experimental procedures

4.1. Experimental model

All studies were performed in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. Female retired breeder Wistar rats ($n=6$, 10–12 months old, 300–450 g) were used. They were anesthetized using halothane (3.5% induction, 0.7% to 1.5% maintenance) in a 2:1 mixture of $\text{N}_2\text{O}/\text{O}_2$ and core temperature was maintained at $36\text{--}37^\circ\text{C}$ throughout all surgical and MRI procedures. Transient focal cerebral ischemia was induced by intraluminal filament occlusion of the right middle cerebral artery (MCA), using a

modification (Chen et al., 1991) of the method originally described by Koizumi et al. (Koizumi et al., 1986) and Longa et al. (Longa et al., 1989). Reperfusion was performed at 2 h by withdrawal of the suture.

4.2. Behavioral tests

A modified neurological severity score (mNSS) and adhesive-removal test (ART) were performed at 1 day, prior to MCAO (baseline) and at 1 day and weeks 2 and 4, and months 2 (the ART only at this time point), 3, 6, 9, and 12 after MCAO. The mNSS includes motor, sensory, balance and reflex tests, as described in detail elsewhere (Chen et al., 2001; Li et al., 2001). Neurological function was graded on a scale of 0–18, with 0 for normal function and 18 for severely impaired. For the ART, time to remove a small adhesive-backed paper dot from each front paw was measured (Schallert and Whishaw, 1984; Shen et al., 2007). Maximum time allowed for each trial was 120 s. The animals were trained for 3 days before surgery. Once they were able to remove the dots within 10 s, they were subjected to MCAO.

4.3. MR imaging

MRI measurements were performed using a 7 Tesla, 20 cm bore superconducting magnet (MagneX Scientific, Inc., Abingdon, UK) interfaced to a Bruker Avance console running Paravision 3.0.2 (Bruker Biospin MRI, Billerica, Massachusetts). The system is equipped with actively shielded gradients capable of producing field gradients of 200 mT/m with rise times of 100 μ s.

After being anesthetized and placing a catheter into the tail vein, the rats were placed in a holder equipped with a nose cone for administering anesthetic gases and ear bars to minimize movement during the MRI scan. A birdcage/surface coil pair was used for radiofrequency (RF) transmission and reception. A 3-plane scout imaging sequence was used to iteratively adjust the position of the animal's head until the central slice was located at the level of the bregma. After completing the setup procedures, a series of MR images were acquired using a 32 mm field of view (FOV). The data series included spin-lattice (T_1) and spin-spin (T_2) relaxation weighted imaging (T_1 WI and T_2 WI, respectively), diffusion-weighted imaging (DWI), magnetization transfer (MT) weighted imaging, and susceptibility weighted imaging (SWI). Animals were studied at 1 day and weeks 1, 2, 3 and 4, and months 3, 6, 9 and 12 post-ischemia.

T_2 measurements—The proton spin-spin relaxation time (T_2) was measured using a standard Carr-Purcell-Meiboom-Gill (CPMG) 2-dimensional Fourier transform (2DFT) multi-slice (13 slices each of 1 mm thickness) multi-echo (6 echoes) MRI sequence. Echo times (TEs) were 20, 40, 60, 80, 100, and 120 ms, and repetition time (TR) was 8.0 s. Images were acquired using a 128×64 matrix.

T_1 and T_{1sat} measurements—Estimates of T_1 were acquired using an imaging variant of the T-one by multiple readout pulses (TOMROP) sequence (Brix et al., 1990; Ewing et al., 1999). Measurements of T_1 in the presence of off-resonance saturation of the bound proton signal (T_{1sat}), an MT related parameter, were also generated using this method. This was done by inserting two continuous wave (CW) RF saturation pulses into the Look-Locker (LL) sequence: the first (4.5 s long) immediately before the inversion pulse and the second (40 ms long) after the signal acquisition. The offset frequency of saturation pulses was 8 kHz, and the rotational frequency of B_1 field was 0.5 kHz. Initially, the longitudinal magnetization was inverted using an 8 ms non-selective adiabatic hyperbolic secant pulse. One phase encode line of 32 small-tip angle gradient echo (GE) images (TE = 7.0 ms) was acquired at 80 ms intervals after each inversion. With this sequence, a single slice T_{1sat} map was obtained in ~12 minutes (TR = 11 s, 128×64 matrix, 2 mm slice thickness). Similarly, a

T_1 map was obtained following the same procedures used for the $T_{1\text{sat}}$ map, but without saturation pulses.

ADC measurements—The ADC was measured using a 2DFT multi-slice spin echo (SE) sequence (13 slices each of 1 mm thickness; 128×64 matrix; TR = 1.5 s; TE = 40 ms) with two 10 ms diffusion-weighted gradient pulses, one on either side of the refocusing 180° RF pulse, as described by Le Bihan et al. (Le Bihan et al., 1986). A series of images was obtained with gradient b-values of 0, 600, and 1200 s/mm² in each of the three orthogonal diffusion sensitizing directions. The trace ADC was estimated using the diagonal elements of the diffusion tensor, as previously described (Schaefer et al., 2000).

SWI measurements—The SWI measurements used a 3-dimensional gradient-echo sequence that is very sensitive to the presence of paramagnetic substances such as iron-compounds. SWI data were acquired using TR/TE = 30ms/10ms, a 25° flip angle, number of averages = 4, 256×256×64 matrix over a 32×32×16 mm³ FOV (125×125×250 μm^3 resolution). Total imaging time was ~33 minutes.

4.4 Histopathology

One year after ischemia, all rats were anesthetized using ketamine (44 mg/kg *IP*) and xylazine (13 mg/kg *IP*) and were sacrificed by transcardial perfusion with saline, followed by perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The intact brain was immediately removed and immersed in fixative overnight. Seven equally spaced coronal sections each of 2 mm thickness were cut using a rodent brain matrix and embedded in paraffin. A series of 6.0 μm thick slices were cut from each section and stained with H&E. The damaged regions were traced using a Global Lab Image analysis system (Data Translation, Marlboro, Massachusetts) and the lesion area was indirectly calculated by subtracting the intact normal tissue area of the ipsilateral side from the area of contralateral hemisphere (Swanson et al., 1990). In this study, the lesions and recovery areas were measured by a trained observer. A series of 6 μm thick sections taken from the center of ischemic lesion, corresponding to coronal coordinates bregma from -1.0 to 1.0 mm, were taken for further analysis using light and fluorescent microscopy (Olympus BH-2, Tokyo, Japan). The histological image was coregistered to MR T_2 images.

To investigate the source of some dark regions that were noted at the borders of the lesion on SWI, the brain sections were also processed for ED-1 (a monoclonal antibody murine macrophage marker) immunostaining and Prussian blue reaction to detect iron. Following blocking in normal serum, slices were incubated with antibodies against ED-1 (dilution, 1:30, AbD Serotec, Oxford, UK) for 1 hour at room temperature. Then the sections were incubated with avidin-biotin-horseradish peroxidase complex and developed in 3'3' diaminobenzidine tetrahydrochloride (DAB). Prussian blue iron staining was performed afterwards. In brief, the slices were incubated for 30 minutes with 2% potassium ferrocyanide (Perls' reagent) in 6% HCl, washed, and counterstained with nuclear fast red. Ferric ions (Fe^{3+}) in the tissue combine with the ferrocyanide resulting in the formation of a bright blue pigment called "Prussian blue" or ferric ferrocyanide.

4.5. MR data processing and statistical analysis

All MR images were reconstructed using a 128×128 matrix except SWI (256×256). The T_2 and ADC maps were produced on a pixel-by-pixel basis using a linear least-square fit. A modified nonlinear optimization procedure was used to produce T_1 and $T_{1\text{sat}}$ maps (Gelman et al., 2001). T_2 maps acquired one year post-ischemia were coregistered to images of the corresponding histology sections using 'Eigentool' image processing and analysis software. The selected slices for all other time points were determined from the one year post-

ischemia T₂ map. The damaged area in the T₂ map was obtained by thresholding the ipsilateral hemisphere T₂ values at 2 times the SD above the mean corresponding contralateral hemisphere T₂ value (excluding LV) and various ROIs were identified.

The 1 day MRI data were referred to as baseline data. The T₂, T₁, T_{1sat}, and ADC maps of the central coronal section of brain were visually coregistered and confirmed by the Eigentool registration tool. The T₂ map of central section was taken to differentiate various ROIs. The ischemia damaged ROIs consisted of core, new lesion and recovering tissue areas (Fig. 2A). The core area for a later time point was the area that appeared to be damaged by thresholding the ipsilateral hemisphere at 1 day post-ischemia and at the time point as well. A “new-lesion” region occurred in some cases, appearing normal at 1 day, but damaged at the later time point. Finally, a third “recovery” region at a later time point was identified as tissue that appeared damaged at 1 day but normal at the later time point. The total lesion area at a later time point was hence divided into two mutually exclusive regions – core and new lesion area, whereas that at 1 day was divided into core and recovery areas. The mean and SD values of the MRI parameters were measured in these selected ROIs and the corresponding contralateral ROIs. All values are reported as mean ± SD. Statistical significance was inferred at $p \leq 0.05$.

For each rat, MRI measurements (T₂, T₁, T_{1sat}, or ADC values) were obtained for the core and recovery regions, as well as their corresponding contralateral regions. Using ADC as an example, the ADC ratio was defined as in the ipsilateral hemisphere ROI ADC measure divided by that of the corresponding contralateral ROIs. For each MRI parameter value or I/C ratio, the following variables were calculated based on our previous experience and are defined as follows:

$$\text{Weighted ADC} = (\text{ADC}_{\text{core}} \times \text{Size}_{\text{core}} + \text{ADC}_{\text{recovery}} \times \text{Size}_{\text{recovery}}) / (\text{Size}_{\text{core}} + \text{Size}_{\text{recovery}}) \quad [1]$$

$$\text{Weighted ADC}_{\text{diff}} = (\text{ADC}_{\text{core}} \times \text{Size}_{\text{core}} - \text{ADC}_{\text{recovery}} \times \text{Size}_{\text{recovery}}) / (\text{Size}_{\text{core}} + \text{Size}_{\text{recovery}}) \quad [2]$$

$$\text{ADC}_{\text{diff}} = \text{ADC}_{\text{core}} - \text{ADC}_{\text{recovery}} \quad [3]$$

To study regional effects (core versus recovery) on MRI parameters, we tested MRI parameter differences at each time between the two regions considering the region size (i.e., weighted MRI parameter difference) using a regression model.

Acknowledgments

The authors thank Jun Xu, Polly A. Whitton, Cindi Roberts and Qing-e Lu for technical assistance. Data were presented in part as an abstract in the 33rd International Stroke Conference 2008, New Orleans, LA, USA. This work was supported by NINDS grants P50 NS23393 (M.C.) and PO1 NS42345 (M.C.).

References

- Brix G, Schad LR, Deimling M, Lorenz WJ. Fast and precise T₁ imaging using a TOMROP sequence. *Magn. Reson. Imaging.* 1990; 8:351–356. [PubMed: 2392022]
- Chen H, Chopp M, Welch KM. Effect of mild hyperthermia on the ischemic infarct volume after middle cerebral artery occlusion in the rat. *Neurology.* 1991; 41:1133–1135. [PubMed: 2067642]

- Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke*. 2001; 32:1005–1011. [PubMed: 11283404]
- Ewing JR, Jiang Q, Boska M, Zhang ZG, Brown SL, Li GH, Divine GW, Chopp M. T_1 and magnetization transfer at 7 Tesla in acute ischemic infarct in the rat. *Magn. Reson. Med*. 1999; 41:696–705. [PubMed: 10332844]
- Gelman N, Ewing JR, Gorell JM, Spickler EM, Solomon EG. Interregional variation of longitudinal relaxation rates in human brain at 3.0 T: relation to estimated iron and water contents. *Magn. Reson. Med*. 2001; 45:71–79. [PubMed: 11146488]
- Gowland, PA.; Stevenson, VL. T_1 : the longitudinal relaxation time. In: Tofts, P., editor. *Quantitative MRI of the Brain: Measuring Changes Caused by Disease*. West Sussex, England: John Wiley & Sons, Ltd; 2003.
- Knight RA, Dereski MO, Helpert JA, Ordidge RJ, Chopp M. Magnetic resonance imaging assessment of evolving focal cerebral ischemia. Comparison with histopathology in rats. *Stroke*. 1994; 25:1252–1261. [erratum appears in *Stroke* 1994 Sep;25(9):1887] discussion 1261–1262. [PubMed: 8202989]
- Koizumi J, Yoshida Y, Nakazawa T, Ooneda G. Experimental studies of ischemic brain edema, 1: a new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. *Jpn. J. Stroke*. 1986; 8:1–8.
- Lansberg MG, Thijs VN, O'Brien MW, Ali JO, de Crespigny AJ, Tong DC, Moseley ME, Albers GW. Evolution of apparent diffusion coefficient, diffusion-weighted, and T_2 -weighted signal intensity of acute stroke. *AJNR: Am. J. Neuroradiol*. 2001; 22:637–644. [PubMed: 11290470]
- Le Bihan D. Looking into the functional architecture of the brain with diffusion MRI. *Nature Reviews Neuroscience*. 2003; 4:469–480.
- Le Bihan D, Breton E, Lallemand D, Grenier P, Cabanis E, Laval-Jeantet M. MR imaging of intravoxel incoherent motions: application to diffusion and perfusion in neurologic disorders. *Radiology*. 1986; 161:401–407. [PubMed: 3763909]
- Li Y, Chen J, Chopp M. Adult bone marrow transplantation after stroke in adult rats. *Cell Transplant*. 2001; 10:31–40. [PubMed: 11294470]
- Liu Y, D'Arceuil HE, Westmoreland S, He J, Duggan M, Gonzalez RG, Pryor J, Crespigny AJ. Serial diffusion tensor MRI after transient and permanent cerebral ischemia in nonhuman primates. *Stroke*. 2007; 38:138–145. [PubMed: 17122422]
- Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke*. 1989; 20:84–91. [PubMed: 2643202]
- Modo M, Beech JS, Meade TJ, Williams SC, Price J. A chronic 1 year assessment of MRI contrast agent-labelled neural stem cell transplants in stroke. *Neuroimage*. 2009; 47 Suppl 2:T133–T142. [PubMed: 18634886]
- Nagaraja TN, Karki K, Ewing JR, Croxen RL, Knight RA. Identification of variations in blood-brain barrier opening after cerebral ischemia using dual contrast-enhanced magnetic resonance imaging and T_{1sat} measurements. *Stroke*. 2008; 39:427–432. [PubMed: 18174480]
- Schaefer PW, Grant PE, Gonzalez RG. Diffusion-weighted MR imaging of the brain. *Radiology*. 2000; 217:331–345. [PubMed: 11058626]
- Schallert T, Whishaw IQ. Bilateral cutaneous stimulation of the somatosensory system in hemidecorticate rats. *Behavioral Neuroscience*. 1984; 98:518–540. [PubMed: 6539617]
- Shen LH, Li Y, Chen J, Cui Y, Zhang C, Kapke A, Lu M, Savant-Bhonsale S, Chopp M. One-year follow-up after bone marrow stromal cell treatment in middle-aged female rats with stroke. *Stroke*. 2007; 38:2150–2156. [PubMed: 17525391]
- Swanson RA, Morton MT, Tsao-Wu G, Savalos RA, Davidson C, Sharp FR. A semiautomated method for measuring brain infarct volume. *J. Cereb. Blood Flow Metab*. 1990; 10:290–293. [PubMed: 1689322]
- Thomas DL, Lythgoe MF, Pell GS, Calamante F, Ordidge RJ. The measurement of diffusion and perfusion in biological systems using magnetic resonance imaging. *Phys. Med. Biol*. 2000; 45:97–138.

- Weber R, Ramos-Cabrer P, Hoehn M. Present status of magnetic resonance imaging and spectroscopy in animal stroke models. *J. Cereb. Blood Flow Metab.* 2006; 26:591–604. [PubMed: 16292254]
- Weber R, Wegener S, Ramos-Cabrer P, Wiedermann D, Hoehn M. MRI detection of macrophage activity after experimental stroke in rats: new indicators for late appearance of vascular degradation? *Magn. Reson. Med.* 2005; 54:59–66. [PubMed: 15968679]
- Wegener S, Weber R, Ramos-Cabrer P, Uhlenkueken U, Sprenger C, Wiedermann D, Villringer A, Hoehn M. Temporal profile of T₂-weighted MRI distinguishes between pannecrosis and selective neuronal death after transient focal cerebral ischemia in the rat. *J. Cereb. Blood Flow Metab.* 2006; 26:38–47. [PubMed: 15988477]

Fig. 1. T₂, T₁, T_{1sat} and ADC maps of a rat brain at post-ischemia time points from 1 day to 1 year. A generic grey scale bar indicating the range from low to high values is shown at the bottom. The values for all these magnetic resonance parameters in lesion areas were higher than that in corresponding topographically matching contralateral areas except for ADC at 1 day.

Fig. 2.

An example of the ischemic core (i_1), recovery (i_2), and new lesion (i_3) regions of interest (ROIs) in the ipsilateral hemisphere is shown on T_2 maps that were acquired at 1 day and 1 year (A). The plots shown below demonstrate the temporal evolution of T_2 (B), T_1 (C), T_{1sat} (D) and ADC (E) parameters for these ROIs (c_1 , c_2 and c_3 represent the corresponding contralateral ROIs). Ischemia damaged tissues in some regions at 1 day were more severely damaged at the later time point, in contrast tissues in some other regions were either recovered or taken up by the ventricles at the later time point; normal tissues in some areas adjacent to the ischemic area at 1 day became damaged later in some cases possibly due to ongoing cell death or changes in the geometry of the injured brain.

Fig. 3.

Temporal evolution of ischemia damaged total lesion and recovery areas (left y-axis with one-sided error bars), and the ratio of ipsi/contra lateral ventricle (LV) areas (right y-axis with two-sided error bars). Note that there are three different scales on the time axis used to make the MRI scan time points equidistant. Mean lesion area was minimum at 1 year post-ischemia whereas mean recovered area was maximum at 1 week. Continuous tissue loss to the LVs and resolution of edema observed at acute time points also affect the values of these areas. Mean value of the ratio of ventricle areas is continuously increased from 1 day to 1 year after stroke at a higher rate at early time points and at lower rate at late time points.

Fig. 4. Hypointense regions were seen in the lesion regions near the ipsilateral ventricle in susceptibility-weighted image (A); macrophages (brown in the Figures) were found in ED-1 immunohistochemistry stained sections magnified 10 $\times$ (B) and 40 $\times$ (C). Panel C is high magnification of box area in panel B. The arrow in Panel C clearly shows colocalization of blue color for iron-positive from Prussian blue and brown color for macrophage-positive from ED-1 staining. The small pink-colored spots represent nuclei. Scale bar is 200 μ m (B) and 50 μ m (C). In panel A, ipsilateral ventricle area of the representative animal is seen much bigger than contralateral ventricle area at 1 year post-stroke.

Table 1

Behavioral tests

Time post-MCAO	Modified neurological severity scores test					Adhesive-removal test ^{a,b}				
	n	Median	IQR ^c	Mean	SD ^d	n	Median	IQR ^c	Mean	SD ^d
1 day	6	11.00	0.75	10.50	1.38	6	120.00	0.00	120.00	0.00
2 weeks	6	6.50	0.75	6.67	1.03	6	95.00	16.50	94.50	18.76
4 weeks	6	6.25	0.88	6.17	1.21	6	73.50	53.00	75.33	34.30
2 months	0	...	...	...	...	6	75.00	32.25	80.50	32.67
3 months	6	6.25	0.88	6.17	1.21	6	65.00	51.00	71.33	35.51
6 months	6	6.50	1.00	6.17	1.17	6	50.00	26.25	58.50	26.71
9 months	6	5.75	0.88	5.42	0.80	6	69.00	43.00	65.50	24.69
1 year	6	5.00	1.50	5.00	0.89	6	36.50	41.75	46.17	26.38

n: total number of animals in the study;

^a measured in seconds;

^b the tab size was made half after 4 weeks to increase the sensitivity of the adhesive-removal test;

^c interquartile range;

^d standard deviation; mean baseline scores before stroke were 0 for modified neurological severity scores test, and less than 10 s for adhesive-removal test.

Table 2

Total lesion MRI values

Time post-MCAO	T ₂ mean (±SD) (ms)		T ₁ mean (±SD) (ms)		T ₁ sat mean (±SD) (ms)		ADC mean (±SD) (10 ⁻⁴ mm ² /s)	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
1 day	91(±12)	55(±5)	1900(±297)	1427(±135)	800(±82)	582(±31)	6.05(±0.56)	7.26(±0.25)
1 week	82(±12)	54(±3)	1640(±161)	1325(±109)	759(±144)	536(±41)	8.38(±1.27)	7.03(±0.16)
2 weeks	111(±20)	54(±3)	1958(±281)	1357(±132)	1207(±332)	538(±41)	12.31(±2.88)	7.26(±0.31)
3 weeks	118(±26)	55(±1)	2036(±385)	1378(±140)	1315(±415)	527(±32)	13.18(±3.40)	7.07(±0.27)
4 weeks	133(±33)	55(±2)	2163(±335)	1408(±134)	1442(±410)	518(±45)	13.74(±3.46)	6.88(±0.42)
3 months	202(±94)	53(±4)	2308(±586)	1315(±116)	1754(±724)	507(±35)	16.17(±5.26)	6.81(±0.25)
6 months	230(±112)	53(±3)	2302(±688)	1307(±181)	1738(±804)	501(±62)	17.66(±4.64)	6.95(±0.41)
9 months	226(±104)	54(±3)	2227(±602)	1228(±206)	1754(±713)	482(±70)	17.35(±4.44)	6.95(±0.18)
1 year	218(±111)	51(±4)	2472(±585)	1364(±143)	1944(±759)	546(±67)	18.61(±5.15)	6.94(±0.29)

Mean (± standard deviation) values of the MRI parameters (T₂, T₁, T₁sat, and ADC) of total lesion areas (containing both core and new lesion areas) and corresponding regions of interest in the contralateral hemisphere of the brain.