Diffusional Kurtosis and Diffusion Tensor Imaging 
Reveal Different Time-Sensitive Stroke-Induced 
Microstructural Changes
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Background and Purpose—Diffusion MRI is a promising, clinically feasible imaging technique commonly used to describe white matter changes after stroke. We investigated the sensitivity of diffusion MRI to detect microstructural alterations in gray matter after sensorimotor cortex stroke in adult male rats.

Methods—The mean diffusivity (MD) and mean kurtosis of perilesional motor cortex were compared with measures in the contralesional forelimb area of sensorimotor cortex at 2 hours, 24 hours, 72 hours, or 25 days after surgery. MD and mean kurtosis were correlated to the surface densities of glia, dendrites, and axons.

Results—Perilesional mean kurtosis was increased at 72 hours and 25 days after stroke, whereas MD was no longer different from contralesional sensorimotor cortex at 24 hours after stroke. There was a significant increase in the density of glial processes at 72 hours after stroke in perilesional motor cortex, which correlated with perilesional MD.

Conclusions—These data support that mean kurtosis and MD provide different but complimentary information on acute and chronic changes in perilesional cortex. Glia infiltration is associated with pseudonormalization of MD in the perilesional motor cortex at 72 hours after lesion; however, this association is absent 25 days after lesion. These data suggest that there are likely several different, time-specific microstructural changes underlying these 2 complimentary diffusion measures. (Stroke. 2015;46:00-00. DOI: 10.1161/STROKEAHA.114.006782.)

Key Words: diffusion tensor imaging • glial fibrillary acidic protein • ischemia • motor cortex • stroke

Each year, ≈795,000 Americans experience a stroke and ≈400,000 will have long-term disability,1 predominantly motor impairments.2 Brain areas that are connected, via overlapping arterial territory and are part of the same neural networks, undergo time-dependent structural and functional changes after ischemia1 and often undergo experience-dependent regeneration.6,7 Perilesional motor areas are involved in spontaneous and experience-driven functional recovery4,8 and treatments that enhance motor recovery result in greater neural plasticity in these regions.5,10 Currently, there are relatively few tools available for clinicians to noninvasively investigate these changes in perilesional motor areas acutely or chronically. The ability to evaluate and understand these time-dependent microstructural changes occurring in the perilesional regions may lead to more individually tailored treatments. Diffusion MRI (dMRI) is a promising and clinically feasible tool, but too little is known about the exact relationships between dMRI and the underlying tissue microstructure across the time span of stroke recovery.

Diffusion tensor imaging and diffusion kurtosis imaging are sensitive to microstructure changes in the brain.13–16 Diffusion tensor imaging assumes water diffusion to be Gaussian and, therefore, is unable to completely characterize tissue microstructure. Diffusional kurtosis imaging is a clinically feasible dMRI method that accounts for diffusional non-Gaussianity, which can reveal more about the heterogeneity and complexity of central nervous system tissue.17–19 and thus is a compliment to standard diffusion tensor imaging. The application of dMRI techniques in surviving perilesional gray matter, previously shown to undergo stroke-related changes, has been under investigated. A clearer understanding of the relationships between perilesional cortical dMRI metrics, tissue biophysics, and morphological alterations after stroke is needed.

In this study, we investigated the sensitivity of dMRI to reveal changes in perilesional remaining motor cortex (MC) acutely and more subchronically after a unilateral, focal endothelin-1 (ET-1)–induced stroke of the forelimb area of the sensorimotor cortex (Fl-SMC). We examined the acute postlesional time point 2 hours after surgery, a time point that

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captures the early stages of ET-1–induced vasoconstriction and reduced blood flow, and 24 hours after ischemia, a time when blood flow has been reported to return to control levels after ET-1–induced stroke.20 Previously, changes in dMRI after a middle cerebral artery occlusion in rats revealed that diffusional kurtosis at 72 hours was elevated while diffusion tensor measures were no longer different from the nonlesional homologous brain region at this time point.14 Thus, we included this subacute time point for comparison across studies, in keeping with recommendations from Stroke Treatment Academic Industry Roundtable.21 Finally, we explored subchronic effects of stroke on dMRI measures 25 days after stroke, in areas known to support recovery of function.5,10–12

Similar to previous reports,16 we found that diffusional kurtosis (mean kurtosis [MK]) continues to be elevated in perilesional areas, in this case the remaining MC, compared with the nonlesional Fl-SMC acutely and subchronically (25 days) after unilateral, SMC lesions. However, the mean diffusivity (MD) of perilesional remaining MC was only significantly elevated acutely during vasoconstriction (2 hours after surgery) and then was no longer different from contralesional SMC at 24 hours, 72 hours, or 25 days after lesion. Our data revealed that an increase in glia infiltration into the remaining perilesional cortex at 72 hours was significantly related to MD in peri-injury cortex. These data suggest that the pseudonormalization of MD early after stroke in the perilesional cortex may be in part because of the increased diffusibility of water in astrocytes.

Materials and Methods

Animals

Long Evans male rats (n=33; 3- to 4-month old) received food and water ad libitum and were kept on a 12:12-hour light:dark cycle. Rats were randomly assigned to 1 of 4 groups that underwent MRI scans before injury (day 0) and at 2 hours, 24 hours, 72 hours, or 25 days after injury using a 7T/30 Bruker BioSpec (Billerica, MA) animal scanner. A 2 shot spin-echo echo planar imaging diffusion sequence with 30 diffusion-encoding directions and 4 b values (0, 650, 1300, and 2000 s/mm²) was used. Other imaging parameters were repetition time/echo time, 4750/32.5 ms, field of view=30 mm×30 mm, matrix=128×128, in-plane resolution=0.23×0.23×1 mm, δ/Δ=5/18 ms, and number of excitations, 2. Nineteen axial slices with no gap were collected with a slice thickness of 1 mm.

Diffusion MRI

MRI scans were acquired before injury (day 0) and at 2 hours, 24 hours, 72 hours, or 25 days after injury using a 7T/30 Bruker BioSpec (Billerica, MA) animal scanner. A 2 shot spin-echo echo planar imaging diffusion sequence with 30 diffusion-encoding directions and 4 b values (0, 650, 1300, and 2000 s/mm²) was used. Other imaging parameters were repetition time/echo time, 4750/32.5 ms, field of view=30 mm×30 mm, matrix=128×128, in-plane resolution=0.23×0.23×1 mm, δ/Δ=5/18 ms, and number of excitations, 2. Nineteen axial slices with no gap were collected with a slice thickness of 1 mm.

Data presented are for MD and MK corresponding to the apparent diffusion and kurtosis coefficient, respectively, averaged over all directions.23 Diffusion and diffusional kurtosis tensors were calculated using diffusion kurtosis estimator, a publicly available in-house software.23 Multislice regions of interest were manually drawn in the (1) infarct core (Figure 1A), (2) homologous region in the contralesional SMC, (3) perilesional layers II/III and V of the remaining MC (Figure 2A), and contralesional layers II/III and V of the Fl-SMC. The perilesional regions of interest were drawn on 3 contiguous MRI slices (1-mm thick) and were inclusive of layers II/III and V.

MD and MK results were analyzed and presented as a ratio of postlesional/prelesional values, allowing for comparison and more comprehensive visual representation of stroke-related changes in dMRI of both hemispheres compared with their prelesion values (Figures 1 and 2). In addition, this allowed us to reduce interanimal differences between groups of animals. Pre- versus postlesion MD and MK results show similar patterns of changes as those of postlesional/prelesional ratios (data not shown). The actual MD and MK values, however, were used to investigate the relationships between all MRI scans. In the acute stroke animals euthanized at 2 hours, 24 hours, or 72 hours, unilateral ischemic lesions were induced via infusion of ET-1 (American Peptide, Inc) into layer V of the left Fl-SMC through 4 holes drilled at 0.5 mm posterior, 2.5 mm anterior, and 3.5 and 4.6 mm lateral to bregma.22 One microliter of ET-1 (0.2 μg/μL in sterile saline) was injected into each hole via a Hamilton syringe (lowered to 1.5 mm DV), at a rate of 1 μL/2 minutes.

For the 25-day postlesion group (these animals were included from a different study), animals received a cocktail of ketamine (110 mg/kg) and xylazine (70 mg/kg). Craniotomy was performed at 0.5 mm posterior and 1.5 mm anterior to bregma and 3.0 to 5.0 mm lateral to midline, and then dura was gently retracted. Four microliters (0.2 μg/μL in sterile saline) of ET-1 was applied directly on the brain surface at 1 μL/min, with a 2-minute wait between each 1 μL of ET-1 application.

After the final application of ET-1 in both sets of surgeries, the brain was left undisturbed for 5 minutes and then the holes or craniotomy was covered with gel film and UV-cured dental acrylic. These coordinates for ET-1 application reliably produce sensorimotor forelimb deficits and cause damage to the forelimb SMC overlap region.10,11,22
perilesional remaining cortex and microstructural changes in glia, dendrites, and axon densities in these regions.

Tissue Processing
After MRI scans at 2 hours, 24 hours, 72 hours, or 25 days after injury, animals were deeply anesthetized with pentobarbital (euthasol, 100 mg/kg, IP) and perfused with 0.1-mol/L phosphate buffer and 4% paraformaldehyde. Six serial rostral to caudal sets of 50-μm coronal sections were produced using a vibratome and stored in cryoprotectant. Three sets of sections were processed for immunohistochemistry to ascertain postinjury morphological changes in the surface density of glia processes, dendrites, and axons in the perilesional MC.

Briefly, as described previously, free-floating sections were processed for immunohistochemistry. Tissue was incubated for 48 hours in one of the following primary antibodies: glial fibrillary acidic protein (GFAP) for glia (1:800 rabbit polyclonal; Dako), microtubule protein 2 (MAP2), for dendrites (1:500 mouse monoclonal; Sigma-Aldrich), and for axons, Pan-Axonal Neurofilament Marker (SMI-312; 1:2000 mouse monoclonal; Covance). Sections were incubated in peroxidase-linked avidin–biotin complex (ABC kit) for 2 hours. Immunoreactivity was visualized using 3,3′-diaminobenzidine with nickel ammonium sulfate intensification. All animals were included in each batch of immunohistochemistry processing and each batch included negative control sections without primary antibody. For the 25-day subset of animals, tissue was only processed for GFAP immunohistochemistry to explore changes in the density of glia, dendrites, and axons with postlesional dMRI metrics. The significance level was α=0.05.

Microstructure Quantification
The cycloid grid intersection method was used to determine the surface density (Sv) of GFAP, MAP2, and SMI-312 immunoreactive processes. For each antibody, we sampled tissue that was represented in 3 adjacent MRI slices that included the Fl-SMC. Although we did not directly coregister regions of interest and immunohistochemistry section, we did carefully select tissue regions based on clear cytoarchitectural features of the tissue and lesion boundaries. Data were obtained in 3 adjacent coronal sections (±600 μm apart), which included perilesional MC and contralesional Fl-SMC (ie, between ±1.2 and −0.26 mm anterior/posterior relative to bregma). Cycloid arcs (ImageJ) were overlaid on light microscopic images, taken at ×100 oil immersion (final magnification, ×1400), of 4 sample regions per hemisphere, 2 adjacent sets (±250 μm) in layers II/III and 2 set in layer V beginning at ±250 μm medial to the lesion core (toward midline). Each immunoreactive process that crossed an arc was counted. The surface density was calculated using the formula $Sv=2(I/L)$, where $I$ is the total number of intersections and $L$ is the sum of the cycloid arc lengths.

Statistical Analysis
All data are reported as averaged group mean with ±SEM. One-way ANOVA was used to test for time point differences using SPSS software. Multiple comparisons were corrected using Bonferroni post hoc analyses to further explore lesion time point differences. Pearson linear correlations were used to determine relationships between changes in the density of glia, dendrites, and axons with postlesional dMRI metrics. The significance level was α=0.05.

Results
MD and MK of Lesion Core and Homologous Contralesional SMC
MD was significantly different in the lesion core compared with the contralesional homologous cortex ($F_{3,29}=22.547; P≤0.01$). Similar to previous reports, a significant reduction in MD in the lesion core was significantly reduced compared with the contralesional homologous cortex at 2 hours ($P≤0.01$) and 24 hours ($P≤0.01$) after stroke (Figure 1B), but there were no longer significant differences at 72 hours and 25 days after lesion.

Similar to previous findings, MK remained significantly elevated across all time points in the lesion core ($F_{3,29}=31.194; P≤0.01$) compared with contralesional SMC (Figure 1C) at 2 hours, 24 hours, 72 hours, and 25 days after lesion ($P≤0.01$). There were no significant differences between postlesional time points.

MD and MK of Perilesional Remaining MC and Contralesional Fl-SMC
At 2 hours after lesion (mean=0.95±0.02), MD was subtly but significantly decreased in remaining perilesional MC compared with nonlesional Fl-SMC (mean=0.99±0.01; Figure 2B; $P≤0.05$). At 24 hours, 72 hours, and 25 days after lesion, perilesional MD was no longer significantly different from the nonlesional Fl-SMC.

However, MK remained elevated in the remaining MC days and weeks after injury compared with the contralesional Fl-SMC. Perilesional MK in the remaining MC was significantly increased compared with contralesional Fl-SMC at 72 hours ($P≤0.05$) and 25 days ($P≤0.05$) after lesion and there was a nonsignificant tendency at 24 hours after lesion ($P=0.054$; Figure 2C).

Surface Density of Glia Processes (GFAP Immunoreactivity)
There was a significant effect of time after injury on the surface density of GFAP-immunoreactive processes in perilesional MC ($F_{3,29}=26.822; P≤0.01$). At 72 hours, there was a
significant increase in GFAP-positive processes compared with contralesional Fl-SMC \((P \leq 0.001)\) and compared with acute (2 hours, \(P \leq 0.01\); 24 hours, \(P \leq 0.01\)) and subchronic (25 days; \(P \leq 0.01\)) postlesional time points (Figure 3B).

In addition, there was a significant increase in the density of glia processes in the nonlesional Fl-SMC at 72 hours compared with 2 hours \((P \leq 0.05)\). In the contralesional SMC, glia infiltration was elevated acutely compared with the subchronic 25-day postlesional time \((p' \leq 0.01)\).

**Surface Density of Dendrites (MAP2 Immunoreactivity)**

There were no significant differences between peri-injury MAP2 surface densities at any acute time point compared with the nonlesional Fl-SMC \((P \geq 0.05)\).

**Surface Density of Axons (SMI-312 Immunoreactivity)**

There were no significant differences between peri-injury surface densities of SMI-312 at any acute time point compared with the nonlesional Fl-SMC \((P \geq 0.05)\).

**Correlation of dMRI With Morphological Characteristics of Perilesional Remaining MC and Contralesional Fl-SMC**

Although MD in the perilesional remaining MC was no longer significantly different from contralesional Fl-SMC, GFAP surface density at 72-hour time point was highly correlated with perilesional MD \((r=0.897; P \leq 0.001)\). The return of MD to pseudonormalized levels acutely after ischemia may be because of microstructure changes related to increased glia infiltration and an increase in water diffusion through these astrocytes. There was also a significant, but weak, correlation overall between MAP2 surface density in the perilesional remaining MC and perilesional MD \((r=0.462; P \leq 0.05\), data not shown).

**Discussion**

These studies provide further evidence that MK and MD reveal different but likely complimentary information about microstructural changes after stroke. Similar to previous findings,\(^4\) MD in the lesion core was reduced at 2 and 24 hours after lesion, but was no longer significantly different from the contralesional SMC at 72 hours or 25 days after lesion. However, MK of the lesion core remained significantly different from contralesional homotopic SMC for weeks after stroke. These findings are similar to those reported after unilateral middle cerebral artery occlusion in rats and likely reflect, as others have reported in different models, changes in water diffusion associated with edema,\(^26\) axon beading,\(^27\) and demyelination.\(^28\) These early changes in MD and MK in the lesion core are also likely because of other, as of yet unidentified microstructural changes. We were unable to address the underlying cause of these changes because there was rarely lesion tissue remaining 72 hours and 25 days after injury.

In these studies, we also sought to determine whether after a focal, unilateral ET-1–induced ischemic insult to the SMC, alterations in microstructure were detectable using dMRI in the perilesional remaining MC, an area known to undergo acute and chronic changes after injury and experience-dependent changes. We observed that in the perilesional remaining MC and perilesional MD \((r=0.462; P \leq 0.05\), data not shown).

**Figure 3.** Surface density of glia. A, Representative \(\times 100\) image of glia density at 72 hours after lesion in the perilesional area (scale bar, 50 \(\mu m\)). B, The surface density of glial processes was increased at 72 hours in the lesion hemisphere \((P \leq 0.001)\) compared with all time points. There was also an increase in glia response at 72 hours compared with 2 hours in the nonlesional hemisphere \((P \leq 0.05)\). In the nonlesional sensorimotor cortex, glia infiltration was elevated at 25 days after lesion compared with all other times \((P \leq 0.01)\). C, Gliarial fibrillary acidic protein surface density at 72 hours time point, when there is the greatest glia infiltration into the perilesional cortex, was highly correlated with perilesional mean diffusivity \((MD; r=0.897; P \leq 0.001)\).
was robustly elevated at 72 hours and was more strongly correlated with MD at this time point. It is possible that the renormalization of MD at 72 hours actually is indicative of glia infiltration at this acute time period. Glia, specifically astrocytes, expresses aquaporin-4 making them highly permeable to water, leading to increased water diffusion. After ischemic injury, glia infiltration can lead to greater edema; when aquaporin-4 is knocked out, there is reduced edema.29 Thus, the increase in glia infiltration in the perilesional MC at 72 hours after stroke is one explanation for why MD is increased (to more normalized levels) at 72 hours after lesion. However, glia are no longer increased 25 days after lesion, and thus other, as of yet unidentified microstructural changes in perilesional cortex are likely responsible for the pseudonormalized MD measures weeks after stroke. Further study is needed on the full time-course of these changes in microstructure and their relationship to dMRI.

The correlation between perilesional MD and surface density of dendritic processes is weak, but may reflect acute lesion-induced dendritic remodeling. Further investigation is needed to understand this relationship. The water permeability rates are unknown in dendritic processes and these rates likely depend on shape and size. Water permeability may be changing after ischemic damage, leading to MD detecting these changes but likely do not capture all of the changes occurring in perilesional microstructure.

Axonal remodeling after stroke has been demonstrated previously,30 is linked to behavioral recovery,30 and has been associated with changes in dMRI measures, primarily in white matter tracts.24 In the present study, we were interested in whether dMRI could capture axonal degeneration in cortical gray matter. However, we found no significant changes after stroke in the density of axons in remaining MC and no significant correlation with dMRI measures. It is possible that dMRI is not sensitive enough to pick up acute stroke changes in axon density. Further studies are needed.

Together these data indicate that diffusional kurtosis imaging measures are sensitive to changes in tissue properties of acute and chronic perilesional remaining MC; however, this is likely not because of alterations in the surface density of glia processes, dendrites, and axons. However, glia infiltration into the remaining perilesional MC may underlie the normalization of diffusion properties measured by MD at 72 hours after lesion, but likely there are also other structural changes responsible for the apparent normalization of MD during the more chronic poststroke period. In summary, this work is one of the first to characterize morphological changes after ET-1–induced ischemic stroke and to relate these findings to changes in dMRI metrics. These data further support that diffusional kurtosis and diffusion tensor measures provide different but complimentary information on acute and chronic changes in perilesional cortex after stroke. There are likely several different microstructural changes underlying these 2 complimentary diffusion measures and we need to further investigate these time-sensitive, region-specific changes.

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Disclosures

Drs Hesper and Jensen are listed on the diffusional kurtosis imaging patent: system, method, and computer accessible medium for providing real-time diffusional kurtosis imaging (US Patent # 8,811,706 B2, August 19, 2014). The other authors report no conflicts.

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拡散尖度画像と拡散テンソル画像は脳卒中による微細構造変化の時間依存的な違いを明らかにする

Diffusional Kurtosis and Diffusion Tensor Imaging Reveal Different Time-Sensitive Stroke-Induced Microstructural Changes

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脳卒中の急性期を対象として、拡散尖度と拡散テンソル画像の比較を行う。本研究では、成体に作製した実験脳卒中モデルを用いて、脳卒中後24時間、72時間、および25日後における微細構造変化的検証を行った。

結果：脳卒中後の微細構造変化は、24時間後および25日後で増加し、MDは24時間後で病変側に減少した。また、MDは25日後で病変側に減少した。


図1

病変コアと病変対側の相関図を示す。A：病変コア（左）と病変対側（右）の間で、MDは縮小が見られた。B：拡散尖度の平均値（MD）は、24時間後で病変側が大きくなる傾向が見られた。

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