Magnetic Resonance in Medicine 45:1126–1129 (2001)

Diffusion Time Dependence of the Apparent Diffusion Tensor in Healthy Human Brain and White Matter Disease

Chris A. Clark,* Maj Hedehus, and Michael E. Moseley

The diffusion time dependence of the brain water diffusion tensor provides information regarding diffusion restriction and hindrance but has received little attention, primarily due to limitations in gradient amplitude available on clinical MRI systems, required to achieve short diffusion times. Using new, more powerful gradient hardware, the diffusion time dependence of tensor-derived metrics were studied in human brain in the range 8–80 ms, which encompasses the shortest diffusion times studied to date. There was no evidence for a change in mean diffusivity, fractional anisotropy, or in the eigenvalues with diffusion time in healthy human brain. The findings are consistent with a model of unrestricted, but hindered water diffusion with semipermeable membranes, likely originating from the extracellular space in which the average extracellular separation is less than 7 microns. Similar findings in two multiple sclerosis plaques indicated that the size of the water diffusion space in the lesion did not exceed this dimension. Magn Reson Med 45: 1126–1129, 2001. © 2001 Wiley-Liss, Inc.

Key words: diffusion; tensor; brain; MRI

In recent years, diffusion tensor imaging (DTI) has emerged as a widely used quantitative magnetic resonance technique for the investigation of structural and orientational changes in brain tissue during development and in pathological states in vivo. The methodology, originally described by Basser et al. (1), requires the acquisition of MRI with diffusion sensitization in at least six noncolinear directions (2). From this series of diffusion-weighted images, the diffusion tensor elements can be estimated. Subsequent diagonalization of the tensor yields the principal direction of diffusion in each voxel. This direction is approximately aligned with the fiber tract axis, passing through the voxel on the basis that water diffusion is greater along white matter fibers than perpendicular to them.

The observation of diffusion anisotropy in white matter can be quantified in terms of indices derived from the tensor, such as the fractional anisotropy (FA) (3) and volume ratio (4). These indices have been shown to be altered in numerous pathologies, including multiple sclerosis (5), schizophrenia (6), and dyslexia (7), reflecting tissue damage or changes in tract morphology at the microstructural level. In addition, the tensor can be used to provide a measure of tissue water diffusion in which the effects of anisotropy are removed and this is referred to as the mean diffusivity (MD) (1).

The diffusion time (t_D) is a parameter determined by the duration (δ) and separation (Δ) of the sensitizing gradients in the DTI sequence such that $t_D = \Delta - \delta/3$ (8). Specifically, this time represents the period over which the diffusion process is observed and is typically about 30 ms for most diffusion imaging studies in human brain. The observed diffusion anisotropy in white matter is commonly interpreted as a greater restriction or hindrance to water diffusion across axonal structures than along them. That is, diffusing water molecules interact more often with the cellular structures in the transverse direction than along the longitudinal direction, in which fewer boundaries are encountered. By reducing the time over which the diffusion process is observed, it may be possible to reduce the degree of interaction with the surrounding cellular structure. Thus, the diffusion time dependence of measured diffusion can be used to gain insight into the nature of restriction, hindrance, and the approximate separation of the restricting boundaries. If water diffusion is assumed to be in a restricted or hindered environment, it may be possible to observe a reduction in the degree of anisotropy and an increase in the mean diffusivity as free diffusion conditions are reached at short diffusion times.

The following study describes an investigation into the diffusion time dependence of tensor-derived parameters in the human brain in vivo. The utilization of powerful gradient hardware available on a clinical MRI system allows diffusion times as short as 8 ms to be achieved. The results of this study are explained in terms of current models for compartmentalized diffusion in the intra- and extracellular space and the presumed predominance of the extracellular component at the *b* values used in this and other DTI studies.

METHODS

DTI was implemented on a GE Signa 1.5 T MRI system equipped with gradients providing a maximum amplitude of up to 40 mT m^{-1}. A quadrature head coil was used for RF transmission and reception of the NMR signal. Diffusion times of 8, 12, 18, 26, 36, 54.5, and 80 ms were obtained using three different diffusion-sensitized echo planar imaging sequences. The diffusion gradient parameters for each sequence are given in Table 1.

For a diffusion time of 8 ms, a series of four gradient lobes of alternating polarity were applied on each side of the 180° refocusing pulse as shown in Fig. 1a. For a diffusion time of 12 ms, two gradient lobes of alternating polarity were applied on each side of the 180° refocusing

Lucas MRSI Center, Stanford, California.

Grant sponsor: NIH; Grant numbers: 1P41RR09784; 1R01NS35959.

*Correspondence to: Chris A. Clark, Ph.D., Dept. of Clinical Neuroscience, St. Georges Hospital Medical School, Cranmer Terrace, London SW17 ORE, UK. E-mail: c.clark@sghms.ac.uk

Received 20 September 2000; revised 15 November 2000; accepted 4 December 2000.

© 2001 Wiley-Liss, Inc.

Table 1
Diffusion Sensitization Parameters; Number of Lobes on Either Side of the 180° Refocusing Pulse, Maximum Gradient Strength (G_{max}), Gradient Duration (δ), Gradient Separation (Δ), and Diffusion Time (t_D)

Number of lobes	G_{max} (mT m^{-1})	δ (ms)	Δ (ms)	t_D (ms)
4	38.3	10.2	11.4	8
2	28.4	16	17.2	11.9
1	32.1	16.2	23.5	18.1
1	32.8	13.2	30.5	26.1
1	33.0	11.2	39.5	35.8
1	32.6	9.2	57.5	54.5
1	34.3	7.2	82.5	80.1

pulse as shown in Fig. 1b and for the other diffusion times a single gradient lobe was applied as shown in Fig 1c.

All images were acquired with a field of view = 24 × 24 cm, image matrix = 128 × 128, 5 mm slice thickness, and TR = 2 sec. For each sequence the *b* factor was set to 700 sec mm^{-2} and the echo time was set to 121 ms. This ensured that the SNR and precision in the diffusion tensor-derived quantitative maps were identical for each sequence (assuming that diffusion does not change substantially with diffusion time). Following an acquisition without diffusion-sensitization (b = 0), diffusion-weighted images were acquired with diffusion gradients applied in 12 directions. This was comprised of six directions [(x, y, 0), (x, 0, z), (0, y, z), (-x, y, 0), (-x, 0, z), (0, -y, z)] and the negative of those six in order to eliminate diffusion-imaging gradient cross-terms (9). The sequences were validated by obtaining DTI of a water phantom at each of the diffusion times from 8 to 80 ms.

Three healthy subjects were scanned. Seven axial slices were selected encompassing the corpus callosum and basal ganglia. DTI was repeated five times and the magnitude reconstructed images averaged for each of the diffusion times studied. Prior to calculation of the diffusion tensor elements, the raw diffusion-weighted images were unwarped to remove distortions caused by eddy currents during the echo-planar readout train using a registration technique (10). The diffusion tensor was then calculated on a pixel-by-pixel basis and diagonalized as described by Basser et al. (1). MD, FA, and eigenvalue maps were then calculated. Regions of interest (ROIs) were positioned in the genu and splenium of the corpus callosum and centrum semiovale in each hemisphere on the MD maps. These regions were then transferred to the FA and eigenvalue maps and the mean values within the ROIs recorded.

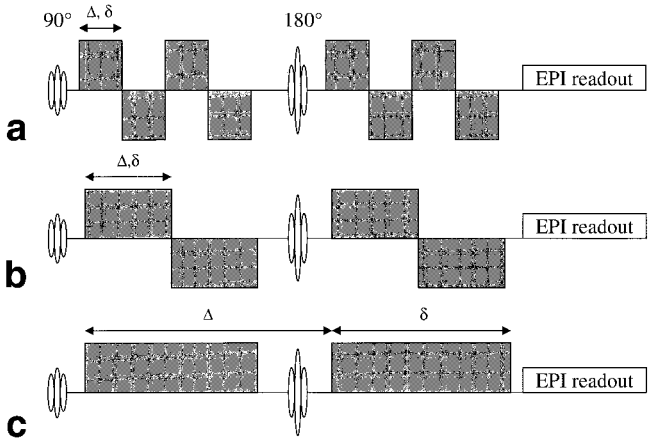

FIG. 1. Schematic pulse sequence diagrams indicating the arrangement and number of diffusion gradient pulses required to achieve a diffusion time of (**a**) 8 ms, (**b**) 12 ms, and (**c**) 18–80 ms. The gradient ramp up and down periods have been neglected. δ represents the gradient duration, Δ represents the gradient separation, and the diffusion time t_D is given by Δ–δ/3.

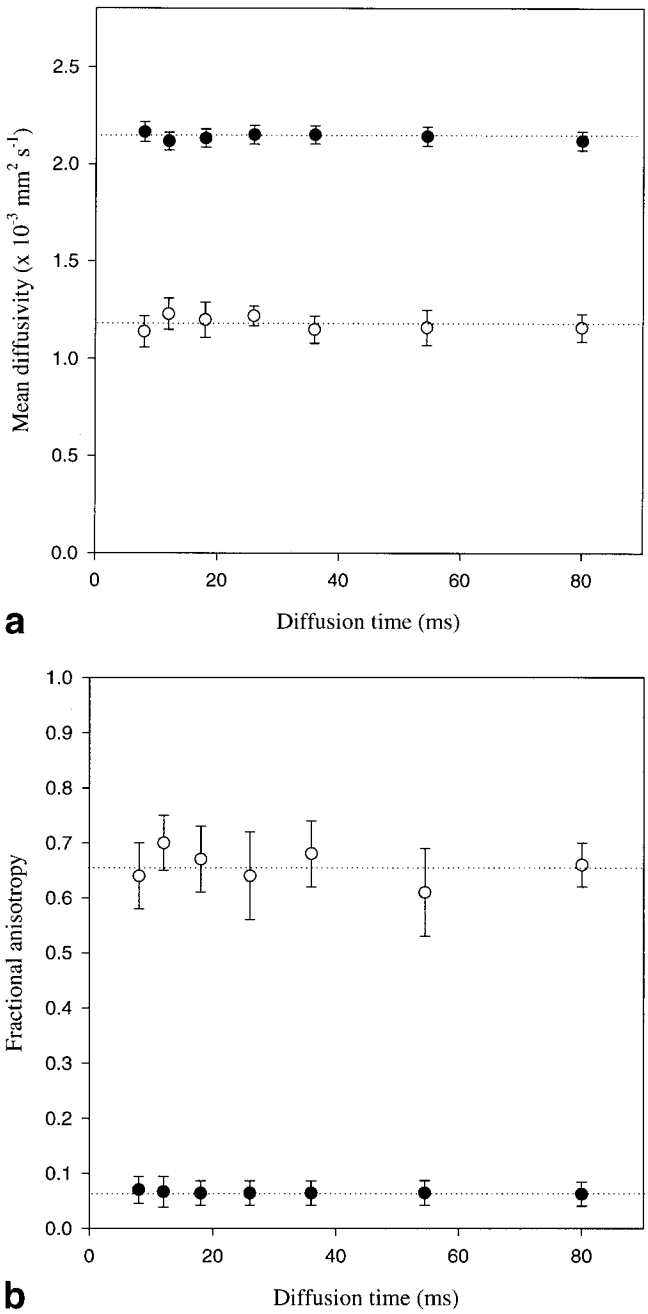

FIG. 2. Diffusion time plots of (**a**) MD and (**b**) FA obtained in a water phantom (black circles) and genu of the corpus callosum in a healthy volunteer (open circles). The error bars represent the standard deviation of the measurement within the ROI.

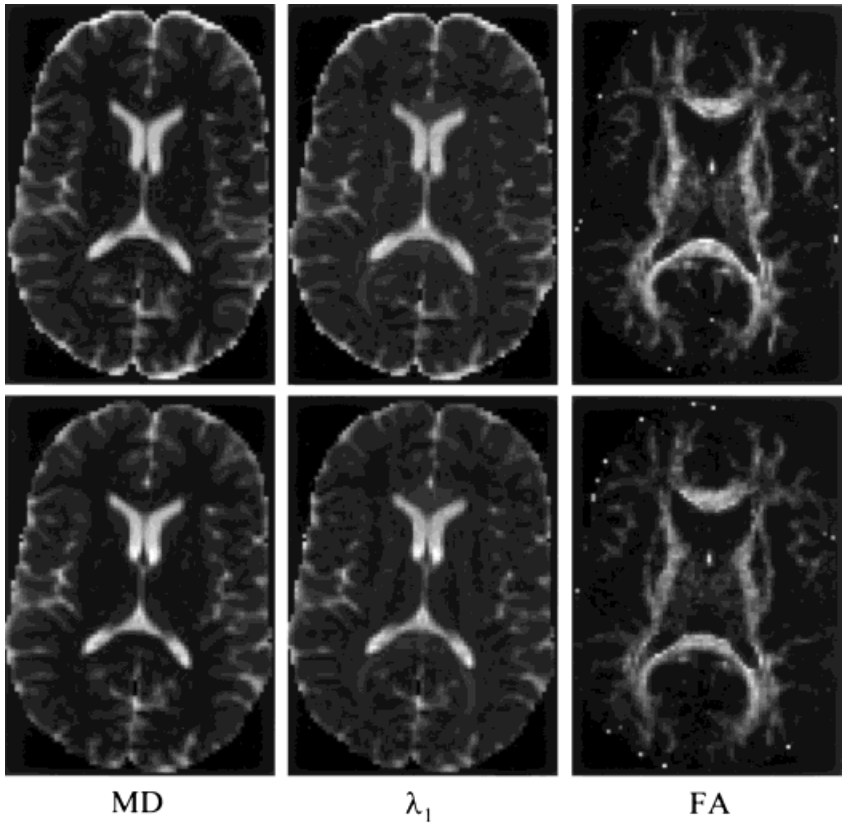

FIG. 3. MD, FA, and λ_1 in normal human brain obtained at the shortest (top) and longest (bottom) diffusion times studied.

DTI was also performed on two patients with clinically definite multiple sclerosis with white matter hyperintensities on conventional T_2-weighted MRI. The diffusion time dependence of MD, FA, and the eigenvalues was examined in two lesions, one in each patient with the lesion ROI delineated on the MD image.

RESULTS

MD and FA obtained in a water phantom at 22°C, with diffusion times between 8 and 80 ms are plotted in Fig. 2a,b (black circles), respectively. As expected, there was no diffusion time dependence in MD, which had an average value over the diffusion times studied of $(2.14 \pm 0.05) \times 10^{-3}$ mm^2 s^{-1}, in close agreement with literature values (11). The FA was 0.06 ± 0.02, indicating essentially isotropic diffusion in the water phantom, which remained constant over all the diffusion times studied.

Typical maps of MD, FA, and the principal eigenvalue (λ_1) in normal human brain are shown in Fig. 3, at the shortest and longest diffusion times studied (top and bottom row, respectively). MD, FA, and the eigenvalues were diffusion time-independent in the genu and splenium of the corpus callosum and in the centrum semiovale in each hemisphere of the three healthy volunteers. Plots of MD and FA against diffusion time in the genu of the corpus callosum for a representative subject are shown in Fig. 2a,b (open circles), respectively. MD, FA, and λ_1 were also found to be diffusion time-independent in the two multiple sclerosis lesions studied.

DISCUSSION

This is the first study to investigate the diffusion time dependence of the diffusion tensor-derived metrics, MD, FA, and eigenvalues in human brain. The results indicate that these parameters do not change significantly over the range of diffusion times between 8–80 ms. This finding is in agreement with the observations of Le Bihan et al. (12) that demonstrated a diffusion time independence in the apparent diffusion coefficients measured along three orthogonal gradient axes in the human brain with diffusion times between 16–79 ms.

Evidence for diffusion time dependence has, however, appeared in the literature (13,14). The key difference between these studies and that of Le Bihan et al. (12) and the present study is that the diffusion times were achieved over a range of b values. Specifically, as the diffusion time was increased the b value was increased concomitantly. It has recently been shown that the water diffusion signal decay is non-monoexponential and is well-described by a biexponential function (15,16) so that the measured diffusion coefficient based on a monoexponential analysis actually decreases as the b value is increased. Unless the b value is maintained at a constant value for all diffusion times, apparent diffusion time dependence may be introduced due to differential sampling of fast and slow water diffusion compartments.

With regard to diffusion compartmentation, the diffusional signal decay in this study, which uses a b value of 700 sec mm^{-2}, is dominated by the fast diffusion compartment. Although the exact origin of the fast diffusion com-

partment has yet to be determined, it has been suggested that it originates from the extracellular space (16). Based on a simple calculation using the Einstein relation for freely diffusing water at 37°C, the mean square displacement is 7 μm for the shortest diffusion time of 8 ms. Given that the smallest dimension of the extracellular space is much less than a micron (17), one would expect to observe hindered or tortuous diffusion and a diffusion coefficient lower than that for free water at the same temperature. This is consistent with the experimental findings in this study, which suggest that the MD reaches an asymptotic value rather than tending to zero at long diffusion times (which would be expected for truly restricted diffusion) (18). It is also likely that significant exchange is taking place during the diffusion time, presumably between the intra- and extracellular space (16). The lack of time dependence is also consistent with the recent observation that background-induced gradients do not play a significant role in determining the apparent diffusion coefficient in human brain (19). Given the typical dimensions of the extracellular space in central nervous system tissue, much shorter diffusion times, submillisecond in duration, may be required to observe a diffusion time dependence.

Demyelination and axonal loss in multiple sclerosis results in an increase in MD and a reduction in FA relative to healthy brain values (5). As a consequence of axonal damage and demyelination, the extracellular space is expanded (20). Thus, diffusion time dependence in the diffusion tensor-derived metrics could potentially indicate the magnitude of such an expansion. However, in the two lesions studied here, no such time dependence was observed. Thus, one may speculate that the expansion of the extracellular space is to no more than 7 μm in these lesions. One might envisage that the extracellular space might double in a lesion with persistent axonal loss and demyelination or edema, but even then diffusion times approaching 1 or 2 ms may be required to observe a diffusion time dependence. Clearly, further studies examining a larger number of lesions are required to generalize these findings.

CONCLUSION

Diffusion tensor-derived metrics were shown to be diffusion time-independent between 8–80 ms in normal human brain and in two demyelinating lesions in two patients with multiple sclerosis. The diffusion characteristics at long diffusion times appear to be more consistent with a model of water spins that are hindered and in exchange during the diffusion time, rather than experiencing complete restriction by cell membranes. Based on a simple calculation using the Einstein relation, one may place an upper limit of 7 μm on the average size of the water diffusion compartment that predominates at b values up to 700 sec mm^{-2} and a diffusion time of 8 ms. Diffusion times that are submillisecond in duration may be required to observe a significant dependence of the diffusion tensor-derived metrics on diffusion time.

REFERENCES

1. Basser PJ, Le Bihan D, Mattiello J. Estimation of the effective self-diffusion tensor from the NMR spin echo. J Magn Reson Ser B 1994; 103:399–412.
2. Basser PJ, Pierpaoli C. A simplified method to measure the diffusion tensor from seven MR images. Magn Reson Med 1998;39:928–934.
3. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative diffusion tensor MRI. J Magn Reson Ser B 1996;111:209–219.
4. Pierpaoli C, Basser PJ. Toward a quantitative assessment of diffusion anisotropy. Magn Reson Med 1996;36:893–906.
5. Werring DJ, Clark CA, Barker GJ, Thompson AJ, Miller DH. Diffusion tensor imaging of lesions and normal appearing white matter in multiple sclerosis. Neurology 1999;52:1626–1632.
6. Foong J, Maier M, Clark CA, Barker GJ, Miller DH, Ron MA. Neuropathological abnormalities of the corpus callosum in schizophrenia: a diffusion tensor imaging study. J Neurol Neurosurg Psychiatry 2000; 68:242–244.
7. Klingberg T, Hedehus M, Temple E, Salz T, Gabrieli JDE, Moseley ME, Poldrack RA. Microstructure of temporo-parietal white matter as a basis for reading ability: evidence from diffusion tensor magnetic resonance imaging. Neuron 2000;25:493–500.
8. Stejskal EO, Tanner JE. Spin diffusion measurements: spin echoes in the presence of a time-dependent field gradient. J Chem Phys 1965;42: 288–292.
9. Neeman M, Freyer JP, Sillerud LO. A simple method for obtaining cross-term-free images for diffusion anisotropy studies in NMR microimaging. Magn Reson Med 1991;21:138–143.
10. Haselgrove JC, Moore JR. Correction for distortion of echo-planar images used to calculate the apparent diffusion coefficient. Magn Reson Med 1996;36:960–964.
11. Tofts PS, Lloyd D, Clark CA, Barker GJ, Parker GJM, McGonville P, Baldock C, Pope JM. Test liquids for quantitative MRI measurements of self-diffusion coefficients in-vivo. Magn Reson Med 2000;43:368–374.
12. Le Bihan D, Turner R, Douek P. Is water diffusion restricted in human white matter? An echo planar NMR imaging study. NeuroReport 1993; 4:887–890.
13. Gates L, Cameron I. Time dependence of water diffusion in human white matter. In: Proc 3rd Annual Meeting ISMRM, Nice, France, 1995. p 355.
14. Horsfield MA, Barker GJ, McDonald WI. Self-diffusion in CNS tissue by volume-selective proton NMR. Magn Reson Med 1994;31:637–644.
15. Mulkern RV, Gudbjartsson H, Westin C-F, Zengingonul HP, Gartner W, Guttman CRG, Robertson RL, Kyriakos W, Schwartz R, Holtzman D, Jolesz FA, Maier SE. Multi-component apparent diffusion coefficients in human brain. NMR Biomed 1999;12:51–62.
16. Clark CA, Le Bihan D. Water diffusion compartmentation and anisotropy at high b values in human brain. Magn Reson Med 2000;44:852–859.
17. Kandel ER, Schwartz JH. Principles of neural science. Amsterdam: Elsevier; 1985. p 92.
18. Tanner JE, Stejskal EO. Restricted self-diffusion of protons in colloidal systems by the pulsed-gradient spin echo method. J Chem Phys 1968; 49:1768–1777.
19. Clark CA, Barker GJ, Tofts PS. An in vivo evaluation of the effects of local magnetic susceptibility induced gradients on water diffusion measurements in human brain. J Magn Reson 1999;141:52–61.
20. Barnes D, Munro PM, Youl BD, Prineas JW, McDonald WI. The longstanding MS lesion. A quantitative MRI and electron microscopic study. Brain 1991;114:1271–1280.