High $b$-value $q$-space diffusion MRI in myelin-deficient rat spinal cords

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Abstract

In this study, we explore the effect of the lack of myelin on the diffusion characteristics and diffusion anisotropy obtained from high $b$-value $q$-space diffusion-weighted MRI ($q$-space DWI) in excised rat spinal cords. Twenty-one-day-old myelin-deficient ($md$) mutant ($N=6$) and control rats ($N=6$) were used in this study. The MRI protocol included multi-slice T1, T2, proton density (PD) MR images and high $b$-value $q$-space diffusion MRI measured perpendicular and parallel to the fibers of the spine. $q$-Space displacement and probability maps, in both directions, as well as displacement anisotropy maps, were computed from the diffusion data. At the end of the MRI protocol, representative spinal cords from both groups were subjected to electron microscopy (EM). The $md$ spinal cords show different gray/white matter contrast in the T1, T2 and PD MR images as compared with controls. In addition, the mean displacement extracted from the high $b$-value $q$-space diffusion data was found to be dramatically higher in the white matter (WM) of the $md$ spinal cords than the controls when diffusion was measured perpendicular and parallel to the fibers of the spine. However, interestingly, at the diffusion time used in the present study, the difference in the WM displacement anisotropies of the two groups was not found to be statistically significant. Myelin was found to have a pronounced effect on the diffusion characteristics of water in WM but less so on the diffusion anisotropy observed at the diffusion time used in the present study.

Keywords: MRI; Diffusion-weighted MRI; $q$-space DWI; Spinal cord; Myelin deficient

1. Introduction

Diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI) are noninvasive tools that are currently used for detecting abnormalities in the central nervous system (CNS) and mapping CNS fiber orientations [1–4]. The use of DWI and, to an even larger extent, DTI relies, in many cases, on highlighting the anisotropic water diffusion in these tissues. Anisotropic water diffusion was observed in CNS tissues such as the brain, spinal cord and optic nerve [5–7], and also in peripheral nerves [8] more than a decade ago. However, despite the many DWI and DTI studies reported to date, the origin and relative importance of different contributors to diffusion anisotropy in these tissues is poorly understood. It seems that this anisotropy originates from the myelin sheath, axonal membrane and neurofibrils, or a combination of them. In the early days, water anisotropy in white matter (WM) was mainly attributed to myelin [9,10]. However, in an important study, Beaulieu and Allen [11,12] showed that diffusion anisotropy of similar magnitude is observed both in nonmyelinated and myelinated nerves, suggesting that myelin is not a necessary determinant for diffusion anisotropy. In addition, a DTI study of the brain of preterm and newborn humans showed that, although myelination is not necessary for observing this anisotropy, myelination does have an effect on the observed diffusion coefficient [13]. In this study, it was found, however, that anisotropy is present before the development of myelination [13].

Gulani et al. [14] studied the diffusion in the spinal cord of myelin-deficient ($md$) rats and concluded that, although myelination is not a prerequisite for diffusion anisotropy, it does influence the magnitude of the observed anisotropy. Song et al. [15] used shiverer mice to monitor the effect of dysmyelination on the observed anisotropy in WM tracts using DTI. They found that the shiverer mice, in which the main observed pathology is the reduction in myelin, have a much lower diffusion anisotropy relative to their wild-type controls, again demonstrating the relative importance of...
myelin to the observed anisotropy in WM [15]. These authors also pointed out that the Beaulieu and Allen model (the long-nosed garfish) may be different from mammalian WM. However, it should be noted that most of these interesting DWI and DTI studies, including some recent ones [9–15], were performed with relatively low $b$ values, sometimes with only two $b$ values and, in many cases, with relatively short diffusion times. In recent years, indications that suggest that high $b$-value $q$-space DWI is sensitive to the integrity of myelin were provided. However, these indications came from spinal cord maturation [16], spinal cord trauma [17], multiple sclerosis patients [18] and patients suffering from vascular dementia [19]. However, all these pathologies involve much more than the mere lack of myelin. This technique was also shown to be sensitive to structural changes caused by cell swelling induced by glutamate [20].

Therefore, the purpose of this study was to evaluate the effect of myelin on the diffusion characteristics of md and control excised rat spinal cords as obtained by high $b$-value $q$-space diffusion MRI. These md rats are X-linked recessive Wistar rat mutants, which show a near-total lack of myelination in their CNS [21]. Here we report the $T_1$, $T_2$ and proton density (PD) MR images as well as high $b$-value $q$-space diffusion MRI, measured both perpendicular and parallel to the fiber direction of the spinal cords of the two rat groups.

2. Materials and methods

2.1. Sample preparation

This study was approved by the ethical committee of the School of Veterinary Medicine of the University of Wisconsin-Madison. Twenty-one-day-old md ($N=6$) and age-matched control ($N=6$) rats were used. The spinal cords were excised and fixed with 4% paraformaldehyde. The spinal cords were inserted into 5-mm NMR tubes with their long axis parallelly to the $z$-direction (the $B_0$ direction) of the magnet and immersed in Fluorinert (Sigma, St. Louis, MO). The temperature in the magnet was maintained at $25.0\pm0.1^\circ C$ throughout the duration of the experiments. At the end of the imaging protocol, several spinal cords of each group were immersed in 2.5% glutaraldehyde solution and processed for electron microscopy (EM).

2.2. MRI experiments

MRI diffusion experiments were performed on an 8.4-T NMR spectrometer (Bruker, Germany) equipped with a micro5 imaging gradient probe capable of producing pulse gradients of up to 190 gauss cm$^{-1}$ in each of the three directions. The protocol included $T_1$, $T_2$ and PD MR images and a series of diffusion-weighted MR images. The multi-slice $T_1$, $T_2$ and PD MR images (five slices of 1.35 mm thickness with a 0.65-mm gap) were acquired using the spin-echo sequence with a field of view of 0.85 × 0.85 cm.
and matrix dimensions of $256 \times 128$ (transformed into a $256 \times 256$ matrix). The repetition and echo times (TR/TE) of the T1, T2 and PD MR images were 700/15, 3000/50 and 3000/11 ms, respectively. Diffusion-weighted images were acquired using the stimulated-echo diffusion imaging pulse sequence with the following parameters: $TR=2000$ ms, $TE=30$ ms, $\delta=2$ ms, $\Delta=50$ ms, field of view of $0.85 \times 0.85$ cm, matrix dimensions of $256 \times 128$ (transformed into a $256 \times 256$ matrix) and three slices of 1.35 mm thickness with a 0.65-mm gap. The diffusion gradient strength, $g$, was incremented from 0 to 50 gauss cm$^{-1}$ in 16 steps giving a maximal $b$ value ($b_{max}$) of $3.53 \times 10^5$ s cm$^{-2}$ and a $q_{max}$ of 426 cm$^{-1}$. In these diffusion MR images, diffusion was measured perpendicular and parallel to the long axis of the spine. The SNRs for the water signals in the WM were typically 24 and 12, for the minimal and maximal $b$ values used, respectively, when diffusion was measured perpendicular to the long axis of the spinal cords.

2.3. Data analysis

The signal decay of water was analyzed using the $q$-space approach [22,23]. Image analysis of the $q$-space data set was performed as previously described [16]. For each pixel, we performed a Fourier transformation of the signal decay with respect to $q$ values, defined as $\gamma g \delta /2\pi$, which produced a displacement distribution profile. Two parameters of the displacement distribution profile, the displacement [calculated from the full-width at half-height (FWHH)] and the probability for zero displacement (given by the height of the displacement profile at zero displacement), were then extracted for each pixel and used to construct displacement and probability maps. It should be noted that all $q$-space displacement values reported herein are 0.425 times the FWHH of the displacement distribution profile. Anisotropy maps were also calculated from the displacement maps in the two orientations for each pixel according to the following equation:

$$\text{Anisotropy} = \frac{\text{Displacement}(x) - \text{Displacement}(z)}{\text{Displacement}(x) + \text{Displacement}(z)}$$  \hspace{1cm} (1)$$

The statistical significance of the differences of the values extracted from the $q$-space analysis of the diffusion data was obtained by Student’s $t$-test.

The regions of interest (ROIs) in the WM and gray matter (GM) used for the quantitative analysis of the mean displacement and the probability for zero displacement are depicted in Fig. 1G. The ROIs used for EM were selected from the same area in the lateral WM for control and $md$ spinal cords.

3. Results

T1, T2 and PD MR images of representative control and $md$ rat spinal cords are shown in Fig. 1. A clear contrast between the white and gray matters (WM/GM) was observed in all three conventional MR images of the control spinal cords (Fig. 1A–C) while no such contrast was observed in the $T_1$ and PD MR images of the $md$ spinal cords (Fig. 1D and F). A WM/GM contrast was observed in the $T_2$-weighted MR images of the $md$ spinal cord (Fig. 1E). However, the observed contrast in the $T_2$-weighted image of the $md$ spinal cord is, in fact, opposite to the contrast observed for the control spinal cord (compare Fig. 1E and B).

Fig. 3. Displacement (A, B) and probability (C, D) maps obtained from the high $b$-value $q$-space diffusion data of an excised spinal cord of a $md$ rat when diffusion was measured (A, C) perpendicular ($x$-direction) and (B, D) parallel ($z$-direction) to the long axis of the fibers of the spinal cord.

Fig. 4. Average mean displacements (given in $\mu$m) in the WM and GM in the spinal cord of the control and $md$ groups when diffusion was measured (A) perpendicular ($x$-direction) and (B) parallel ($z$-direction) to the fibers of the spine.
The mean transverse areas of the control and md spinal cords, which were extracted from these MR images, were $4.7 \pm 0.5$ and $3.7 \pm 0.5$ mm$^2$, respectively.

Fig. 2 shows the displacement and probability maps extracted from the high b-value q-space DWI data of a representative control spinal cord, when diffusion was measured perpendicular (Fig. 2A, C) or parallel to the fibers of the spine (Fig. 2B, D). Fig. 3 shows the same diffusion data for a representative md spinal cord. Indeed, the WM/GM contrast is observable, to a different extent, in all the q-space DWI maps of both spinal cords. These figures also show that the mean displacements in the GM are larger than those in the WM when the diffusion is measured perpendicular to the long axis of the spinal cord, and smaller in the parallel direction in both the control and md spinal cords. The probability for zero displacement, however, was found to be higher in WM than in the GM when the diffusion was measured perpendicular to the long axis of the spinal cord and lower when diffusion was measured parallel to the long axis of the spine for both rat spinal cords. These displacement maps it is also clear that, in the control spinal cords, the WM/GM contrast was more pronounced when diffusion was measured perpendicular to the fibers of the spinal cords. For the md spinal cord it appears that the differentiation between WM and GM is not very different for both directions.

Fig. 4A and B depicts the mean displacements extracted for ROIs in the WM and GM in the spinal cords of the control and md groups (see Fig. 1G), when the diffusion was measured perpendicular (x-direction) and parallel (z-direction) to the fibers of the spine. We found a significant increase in the mean displacements of the WM of the md spinal cords as compared with the control when diffusion was measured perpendicular and parallel to the fibers. However, the difference between the two groups was much more significant in the WM when diffusion was measured perpendicular to the direction of the fibers of the spine.

The displacement anisotropy maps of a representative control and md spinal cords, which were calculated on a pixel-by-pixel basis using Eq. (1), are shown in Fig. 6A.
4. Discussion

This study shows that the lack of myelin significantly affects the diffusion characteristics of WM in the excised spinal cord. However, the present study shows that the lack of myelin results in somewhat smaller anisotropy in WM, but these changes, under the experimental conditions used in this study, were found to be statistically insignificant when compared with the anisotropy found in their age-matched controls. The lack of myelin resulted in an increase in the mean displacement in both directions. The increase was somewhat larger for diffusion measured perpendicular to the fibers of the spinal cord. However, the overall changes in anisotropy between the two groups were not statistically significant. The EM images shown in Fig. 7 clearly demonstrate that the 21-day-old age-matched control spinal cords had much more myelin than the md spinal cords. According to these results, one may conclude that myelin is not a primary determinant of the observed diffusion anisotropy of water in the WM of the spinal cord. However, several issues should be taken into consideration and discussed before such a generalized statement is made. First, because of the difficulty in bringing the X-linked recessive mutant md rats to maturation, the reported diffusion experiments were performed on 21-day-old rats. Indeed, the WM anisotropy for the md rat spinal cords was nearly zero. However, the anisotropy of the age-matched control was not very high either and was found to be in the range of 0.13±0.02 au. This is consistent with a previous study in which the effect of age on the appearance of q-space displacement and probability maps of fixed rat spinal cords was reported [16]. From this study, it is clear that at 21 days, the rat spinal cord does not have the diffusion characteristics of a mature spinal cord [16]. It is therefore anticipated that if we could perform these experiments on more mature rats the differences between the two groups would have been statistically significant because of the higher anisotropy that would have been found for the mature controls. This is despite the fact that the control spinal cords of the 21-day-old rats had significant myelination relative to the md-rat spinal cords as depicted in the EM images shown in Fig. 7. The second issue that needs to be addressed is the fact that the diffusion time in this study was only 50 ms. It was recently found that, even in control mature spinal cords, the diffusion time had a dramatic effect on the diffusion anisotropy and even on the GM/WM contrast [24]. In that study, it was shown that, indeed, water anisotropy in spinal cord WM increases with the increase in diffusion time [24]. This occurs due to the nearly linear increase in the mean displacement with the square root of the diffusion time when diffusion is measured parallel to the fibers and the much smaller increase when diffusion is measured perpendicular to the long axis of the spinal cord. It is therefore anticipated that, at longer diffusion times, the difference in the water diffusion anisotropy of the WM of the two groups will indeed be statistically different. Such experiments are underway and will be reported in due course.

A third issue is the fact that this study was performed on excised formalin-fixed spinal cords and not computed in vivo. One can always question the relevance of in vitro studies to the in vivo situation. However, there are now several recent comparative studies regarding CNS water diffusion in general and diffusion anisotropy in particular [25,26] which demonstrated that, indeed, diffusion anisotropy is not significantly affected by fixation and that the in vitro results are consistent with the in vivo results obtained under the same experimental conditions. In conclusion, we have demonstrated, using md rat spinal cords, that the lack of myelin has a pronounced effect on the diffusion characteristics of water in WM as obtained from high b-value q-space diffusion MRI. However, only a...
limited effect was observed on the diffusion anisotropy which was attributed partially to the fact that the control group comprised immature rats and the fact that the diffusion time in these diffusion experiments was not very long. Extensive studies regarding the effect of experimental diffusion parameters on the diffusion characteristics and diffusion anisotropy in md rat spinal cords are underway and will be reported in due course.

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