Axon Diameter and Intra-Axonal Volume Fraction of the Corticospinal Tract in Idiopathic Normal Pressure Hydrocephalus Measured by Q-Space Imaging

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Abstract

**Purpose:** Previous studies suggest that compression and stretching of the corticospinal tract (CST) potentially cause treatable gait disturbance in patients with idiopathic normal pressure hydrocephalus (iNPH). Measurement of axon diameter with diffusion MRI has recently been used to investigate microstructural alterations in neurological diseases. In this study, we investigated alterations in the axon diameter and intra-axonal fraction of the CST in iNPH by q-space imaging (QSI) analysis.

**Methods:** Nineteen patients with iNPH and 10 age-matched controls were recruited. QSI data were obtained with a 3-T system by using a single-shot echo planar imaging sequence with the diffusion gradient applied parallel to the antero-posterior axis. By using a two-component low-q fit model, the root mean square displacements of intra-axonal space (= axon diameter) and intra-axonal volume fraction of the CST were calculated at the levels of the internal capsule and body of the lateral ventricle, respectively.

**Results:** Wilcoxon’s rank-sum test revealed a significant increase in CST intra-axonal volume fraction at the paraventricular level in patients (p<0.001), whereas no significant difference was observed in the axon diameter. At the level of the internal capsule, neither axon diameter nor intra-axonal volume fraction differed significantly between the two groups.

**Conclusion:** Our results suggest that in patients with iNPH, the CST does not undergo irreversible axonal damage but is rather compressed and/or stretched owing to pressure from the enlarged ventricle. These analyses of axon diameter and intra-axonal fraction yield insights into microstructural alterations of the CST in iNPH.

Introduction

Idiopathic normal pressure hydrocephalus (iNPH) is a clinical entity of unknown cause and is characterized by the triad of gait disturbance, cognitive deterioration, and urinary incontinence [1]. It is also associated with ventricular enlargement, flattening of high-convexity sulci, and periventricular T2-weighted image hyperintensity in the absence of elevated cerebrospinal fluid (CSF) pressure [2]. Gait disturbance is the most frequent symptom of iNPH [3] and is treated by CSF shunting [4]. Although the etiology of gait disturbance in iNPH is not completely understood, a plausible explanation is that the corticospinal tract (CST) is distorted by expansion of the lateral ventricles [1,5,6].

Diffusion tensor imaging (DTI) has been applied to neurological and psychological diseases and is useful to detect brain abnormalities that can not be recognized by conventional T1- or T2-weighted images [7,8]. Previous studies conducted with DTI revealed increases of fractional anisotropy (FA) and axial diffusivity values in the CST in patients with iNPH [9–17], which tended to return to normal after placement of a ventriculoperitoneal (VP) shunt [9–12]. The increases in FA and axial diffusivity have been suggested to result from ventricular enlargement that mechanically compresses the tract and yields more directional water diffusion along it. Diffusion MRI is expected to become a non-invasive method for diagnosing iNPH and predicting the response to surgery [12,17].

Q-space imaging (QSI), a diffusion MRI technique that does not assume that the displacement probability of diffusing water molecules has a Gaussian distribution, can provide quantitative tissue architecture information at cellular dimensions [18–22]. Recently, analysis of axon diameter of neural fibers by using diffusion MRI is becoming a topic for investigation of microstruc-
tural alteration in neurological disease [23–28], although assessment of axonal architecture usually requires high gradient amplitudes and long scanning times, which are not clinically applicable. A two-component low-q fit model for QSI analysis, proposed by Ong et al. [29], enables measurement of the axon diameters of neural fibers with a reasonable scanning time. Briefly, QSI provides a molecular displacement probability density function (PDF), which reflects the axonal architecture, such as axon membranes and myelin sheath acting as barriers to diffusing molecules. In the white matter, the dominant diffusion barrier is the axonal membrane, and the spacing between barriers can be regarded as mean axonal diameter [19]. A two-component low-q fit model used in this study has the following two merits; 1) it accounts for signal from extra- and intra-axonal spaces and has better correlations with pathological findings than a single-component model, 2) it does not require very high gradient amplitudes [29]. The limitation of this method is that it requires prior knowledge of the fiber orientation, because the diffusion gradient must be applied perpendicular to the fiber direction.

The purpose of this study is to investigate alterations in the axonal architecture of the CST in patients with iNPH by using a two-component low-q fit analysis of QSI.

**Materials and Methods**

**Ethics Statement**

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Juntendo University Hospital and all persons gave their written informed consent prior to their inclusion in the study.

**Patients**

Nineteen patients with iNPH (10 males and 9 females; 74.3±6.2 years old) and 10 age-matched control subjects (3 males and 7 females; 75.8±5.2 years old) were recruited. Diagnosis of iNPH was made according to the diagnostic criteria of probable iNPH [30]. Those who had a history of neurological disease or any significant findings (as observed on routine MR images) that might affect the brain were excluded. Normal control subjects were required to be >60 years of age and have no neurological or psychological symptoms, history of neurological diseases, or apparent abnormalities observed on conventional MR images.

**QSI data acquisition and processing**

QSI data were obtained with a 3-T unit (Achieva, Philips Healthcare, Best, The Netherlands) by using a single-shot echo planar imaging (EPI) sequence. The patient was positioned so that the anterior commissure–posterior commissure (AC-PC) line is parallel to the scanner’s x-y plane. The scan parameters were: repetition rate/echo time (TR/TE) = 4500/99 ms, field of view (FOV) = 240×240 mm², matrix size = 96×96, slice thickness = 5 mm, 10 axial sections including the level of internal capsule, number of excitations (NEX) = 2, half-Fourier factor = 0.667, 16 b-values (0, 1000, 2000, … 15000 s/mm²), applied in sequential order, and acquisition time = 828 s. The gradient duration (Δ) and time between the two leading edges of the diffusion gradient (Δ) were 39.3 and 48.7 ms, respectively. We did not apply any distortion corrections, because correction was difficult especially at high b values and resulted in severe signal defect in some of the patients.

The two-component low-q fit method for axon diameter analysis necessitates a diffusion gradient perpendicular to the fiber tract to be measured. Ideally, the appropriate direction of diffusion gradient can be determined in each patient by performing tractography of the CST, though it is not realistic in clinical examinations. Instead, we applied diffusion gradient parallel to the antero-posterior axis of the scanner’s coordinate system, as the known course of CST [32,33] is substantially perpendicular to the scanner’s x-y plane. Examples of the acquired images are shown in Figure 1. By applying the diffusion gradient parallel to the antero-posterior axis, the CST could be identified as a hypertensive tract running from the precentral gyrus to the cerebral peduncle through the posterior limb of the internal capsule [31]. Each ROI was placed manually so that it includes the bright-line appearing CST, using b = 1000–4000 s/mm² images. The cranial and caudal sections were also used as references for continuity of the tract. Measurements were performed at two levels. The first section was selected so that it contains the posterior limb of the internal capsule. The second one was selected as two or three sections cranial to the first one, where the CST runs closest to the lateral ventricle. By using in-house software developed in Matlab (R2011b; MathWorks, Natick, MA, USA), the root mean square displacements (RMSDs) of the intra-axonal space (axon diameter) and intra-axonal volume fraction of the CST were calculated by fitting the echo attenuations (normalized to the maximum value at the q = 0) to equation (1) with a nonlinear least squares algorithm: \[ E(q) = (1 - f_i) \exp(-2\pi^2 q^2 Z_R^2) + f_i \exp(-2\pi^2 q^2 Z_Q^2) \] where \( f_i \) is the relaxation-weighted intra-axonal volume fraction, and \( Z_R \) and \( Z_Q \) are the RMSDs of diffusing molecules in the extra- and intra-axonal spaces, respectively.

**Statistical analysis**

Statistical analyses were performed by using JMP software (ver. 10.0.2; SAS Institute Inc. Cary, NC, USA). The axon diameter and intra-axonal volume fraction values of the CST from both hemispheres were compared between the patients and controls. To minimize type I errors with multiple comparisons, Bonferroni’s correction was applied. The significance level (\( p = 0.05 \)) was therefore reduced to an adjusted \( p \) level of 0.006.

**Results**

Excellent fitting was obtained in all ROIs (\( R^2 > 0.95 \)). Shapiro-Wilk’s test was performed to test the hypothesis that the data satisfied Gaussian distribution (the significance level was set at \( p = 0.05 \)). As it was revealed that the assumption of Gaussian distribution was not satisfied in the measurements of axon diameter at the internal capsule level (\( p = 0.004 \)), Wilcoxon’s rank-sum test was used for the following group analyses. At the paraventricular level, the CST intra-axonal volume fraction was significantly higher in patients with iNPH than in the controls (right, 0.43±0.04 for the controls, 0.53±0.05 for the patients, \( p = 0.0002 \); left, 0.43±0.06 for the controls, 0.54±0.06 for the patients, \( p = 0.0005 \)), whereas no significant difference was observed in the CST axon diameter. At the level of the internal capsule, no significant differences were observed between the two groups in either axon diameter or intra-axonal volume fraction (Table 1, Figs. 2, 3). There were no statistically significant differences in ROI sizes between the patients and the controls (Student’s t-test; the internal capsule level, 43.6±4.8 mm² for the controls and 43.3±7.3 mm² for the patients, \( p = 0.83 \); the paraventricular level, 43.0±9.6 mm² for the controls and 41.9±7.4 mm² for the patients, \( p = 0.66 \)).

**Discussion**

A two-component low-q fit analysis of QSI revealed that the CST intra-axonal volume fraction in areas near the ventricles was...
increased in iNPH patients compared with controls, whereas the CST axon diameter was unaltered. Neither CST axon diameter nor intra-axonal volume fraction differed significantly at the level of the internal capsule. Our results are in line with previous DTI studies showing increased diffusion anisotropy of the CST in iNPH, presumably due to compaction of neuronal fibers [9–17]. The increase in CST intra-axonal volume fraction was limited to areas near the lateral ventricle in our study, suggesting that it results from compression by the enlarged ventricles. The unaltered axon diameter of the CST suggests that the iNPH patients involved in this study did not have irreversible axonal damage of the CST.

The exact pathogenesis of the gait disturbance in iNPH is not entirely understood. Though our results suggest that the axons are densely packed in the CST with reduced extra-axonal space, there is no readily available explanation why such situation results in the characteristic gait disturbance in iNPH. A classical hypothesis that remains plausible is that the CST is compressed and/or deformed because of enlargement of the lateral ventricles [1,5,6]. Other pathological changes observed in the brains of patients with iNPH, such as ischemia and gliosis due to transependymal diapedesis of the CSF, may to some extent be related to gait disturbance [32–36]. However, the reversibility of symptoms after shunt surgery even after a long period suggests that irreversible axonal damage is unlikely to be the sole cause of gait disturbance [4]. The “compression hypothesis” is also supported by the fact that the fibers of the legs are closest in proximity to the lateral ventricles [37–39]; this explains why gait disturbance is the most prominent neurological feature in iNPH. Moreover, a detailed tract-specific analysis of the CST demonstrated that the increase in FA was limited to areas near the lateral ventricle [15], an observation consistent with CST compression by the ventricular enlargement.

Analyses of the axon diameter and axon density by using diffusion MRI could have a significant impact on our understanding of white matter architecture and connectivity, neuroanatomical changes that occur in white matter disorders, and changes that occur in white matter during normal and abnormal development. These indices are more straightforward and easier to interpret than other diffusion metrics, such as the mean diffusivity, fractional anisotropy, directional diffusivity, and directional kurtosis, each of which must be interpreted in combination with one or more of the others to understand the microstructural

| Table 1. Results of the measurement of CST axon diameter and intra-axonal volume fraction. |
|-----------------------------------------------|
| controls | iNPH patients |
| right | left | right | left |
| Internal capsule level |
| axon diameter (µm) | 2.07±0.35 | 2.00±0.31 | 2.00±0.53 | 2.16±0.28 |
| intra-axonal volume fraction | 0.47±0.05 | 0.46±0.06 | 0.49±0.09 | 0.53±0.08 |
| Paraventricular level |
| axon diameter (µm) | 2.09±0.12 | 2.04±0.14 | 2.10±0.16 | 2.11±0.15 |
| intra-axonal volume fraction | 0.43±0.04 | 0.43±0.06 | 0.53±0.05 | 0.54±0.06 |

doi:10.1371/journal.pone.0103842.t001
changes [40,41]. Because axon diameter determines conduction velocity, this metric and the axon density provide information about the role and performance of white matter pathways [42–44]. Axon diameter analysis would also provide a means of testing hypotheses that assume changes in the diameter distribution in diseases such as amyotrophic lateral sclerosis [45], multiple sclerosis [46], and autism [47].

The present results need to be interpreted carefully and hopefully validated by more dedicated experiments, because the short gradient pulse (SGP) approximation (Δ > δ) was not satisfied in this study. In principal, SGP approximation needs to be fulfilled for accurate compartment size measurement by QSI. However, with clinical scanners, high q-values can only be obtained with long diffusion gradient pulses because of the relatively weak gradients [20,48,49]. The previous experimental studies reported that the diffraction minima is pushed towards higher q values and the extracted compartment size becomes smaller than the real size when the SGP approximation is violated (Δ/δ~1) [50]. Though the situation becomes more complicated when there are more than two compartments [50], we speculate that the intra-axonal volume fraction in this study is larger than the real value, as the echo attenuation curve vs q values shifts to the right. Mathematical calibration with the ideal Δ/δ settings [50], or the use of a double-pulsed gradient sequence [51], may be useful to overcome this issue.

The other limitations to this study include the following. First, interpretation of the increase in intra-axonal volume fraction is somewhat ambiguous as it is not directly equal to axonal density. Though we speculate the increased intra-axonal volume fraction reflects that the neural fibers are densely packed with reduced extra-axonal space, other conditions, such as changes in the distribution of axon diameter (i.e., increase of large- and small-diameter axons with few middle-sized axons), may yield similar results. In addition, the intra-axonal volume fraction obtained by the two-component low-q fit method is not completely proven to correlate with that obtained from pathological analyses [29]. Second, the determination of diffusion gradient direction was approximative, and it could have been slightly different from what it should be (perpendicular to the CST). Also, previous DTI studies in iNPH have typically reported increased FA in the CST and decreased FA in the corpus callosum, suggesting regionally dependent microstructural alterations [9,11,13,15]. Therefore, our results require validation by an orientationally invariant method for measuring the axon diameter [25,54]. Lastly, owing to the small sample size and lack of post-operative imaging, the clinical relevance of the QSI measures, such as in monitoring the effect of surgery or pre-operatively predicting the response to surgery, could not be established.

Conclusions

In this study, an analysis of axon diameter and intra-axonal volume fraction demonstrated that in patients with iNPH, the
CST is compressed by the ventricular enlargement but does not undergo irreversible axonal damage. The axon diameter and intra-axonal volume fraction obtained by QSI yield insights into microstructural alterations in iNPH. Their potential use in predicting the response to surgery or in post-operative monitoring requires further investigation.

Figure 3. Boxplot comparing the CST axon diameter and intra-axonal volume fraction between the controls and iNPH patients. Statistical analyses revealed a significant increase in CST intra-axonal volume fraction at the paraventricular level in the patients, whereas no significant difference was observed in the axon diameter. At the level of the internal capsule, neither axon diameter nor intra-axonal volume fraction differed significantly between the two groups. * The significance level was set at p = 0.006 (Bonferroni’s correction for multiple comparison).

doi:10.1371/journal.pone.0103842.g003
51. Shemesh N, Ozarslan E, Basser PJ, Cohen Y (2009) Measuring small compartmental dimensions with low-q angular double-PGSE NMR: The effect of experimental parameters on signal decay. Journal of Magnetic Resonance 198: 15-23.

52. Kunimatsu A, Aoki S, Masutani Y, Abe O, Mori H, et al. (2003) Three-dimensional white matter tractography by diffusion tensor imaging in ischaemic stroke involving the corticospinal tract. Neuroradiology 45: 532-535.

53. Yamada K, Kizu O, Kukita T, Iso H, Matsushita S, et al. (2007) The pyramidal tract has a predictable course through the centrum semiovale: a diffusion-tensor based tractography study. Journal of Magnetic Resonance Imaging 26: 519–524.

54. Barazzoni D, Jones D, Assaf Y (2011) AxCaliber 3D. Proc Int Soc Magn Reson Med 19: 76.