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Quantitative temporal changes in DTI values coupled with histological properties in cuprizone-induced demyelination and remyelination

Ryutaro Yano [a, b], Junichi Hata [a, c, d], Yoshifumi Abe [b], Fumiko Seki [a, c, d], Keitaro Yoshida [b], Yuji Komaki [a, d], Hideyuki Okano [a, c], Kenji F. Tanaka [b, *]

[a] Department of Physiology, Keio University School of Medicine, Japan
[b] Department of Neuropsychiatry, Keio University School of Medicine, Japan
[c] Laboratory for Marmoset Neural Architecture, RIKEN Brain Science Institute, Japan
[d] Live Imaging Center, Central Institute for Experimental Animals, Japan

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ABSTRACT

Diffusion tensor imaging (DTI) is widely used to evaluate microstructural variations in brain tissue. In particular, fractional anisotropy (FA), reflecting the magnitude and orientation of anisotropic water diffusion, allows us to detect pathological events in white matter. An *ex vivo* DTI study coupled with histological assessment is an efficient strategy to evaluate the myelination process, i.e. demyelination and remyelination. The relationship between DTI values and myelin content or the individual cellular components such as oligodendrocytes, microglia, and astrocytes during both processes of demyelination and remyelination are not well-understood. To address this issue, we employed a cuprizone-inducible demyelination mouse model. Demyelination can be induced in this model during cuprizone exposure and termination of cuprizone exposure induces remyelination. We fed the mice cuprizone-containing chow for 4 weeks and then normal chow for an additional 4 weeks. The *ex vivo* DTI was performed to evaluate the white matter profiles observed by FA, mean diffusivity (MD), and radial diffusivity (RD) at both demyelinating and remyelinating time points, and then we evaluated histological properties at the same time points. The results indicated a gradual FA decrease during the cuprizone treatment (0, 2, 3, 4 weeks). A lower peak was seen at 1 week after the normal chow was resumed, with recovery to baseline at 2 and 4 weeks. MD and RD showed an opposing pattern to that of FA. These DTI values were positively or negatively correlated with myelin content regardless of the status of the white matter. The RD value was more sensitive to myelination status than FA and MD. We have clarified the temporal changes in the DTI values coupled with histological properties over both the demyelination and remyelination processes.

1. Introduction

Magnetic resonance imaging (MRI) is a technique to non-invasively visualize the structural and functional information of brain tissue by detecting biological water molecules. In particular, diffusion tensor imaging (DTI) is a structural MRI technique to detect the diffusion of water molecules, which is greatly influenced by tissue microstructure (e.g., cell membranes, myelin, fibers) (Basser et al., 1994a, 1994b; Le Bihan et al., 2001; Mori and Zhang, 2006). Fractional anisotropy (FA) is a computed DTI value, which reflects the magnitude and orientation of anisotropic water diffusion. It is widely applied to estimate white matter structure (Feldman et al., 2010; Thomason and Thompson, 2011). The FA value is higher in brain regions having abundant and aligned axonal fibers such as white matter and is lower in regions with scarce and misaligned fibers such as gray matter (Basser et al., 1994b; Laitinen et al., 2015). Furthermore, the rotational and invariant DTI parameters of mean diffusivity (MD), which is the average diffusivities over the 3 axes of the diffusion tensor, and radial diffusivity (RD), which represents the diffusivity perpendicular to the main axial direction, are also applicable to the measurement of microstructure or axonal integrity (Alexander et al., 2007; Feldman et al., 2010).

Although FA maps can be obtained for human brains, it is difficult to estimate how much the FA value reflects the degree of

* Corresponding author. 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan.
E-mail address: kftanaka@keio.jp (K.F. Tanaka).

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myelination because the access to human brains for histological validation is limited. If demyelination animal models are used, the brain can be histologically analyzed immediately after the scan and the state of myelinated fibers can be evaluated (Zhang et al., 2007). Among experimental animal models of demyelination, the cuprizone-induced demyelination mouse model can strictly separate demyelination and subsequent remyelination (Matsushima and Morell, 2001).

Cuprizone is cytotoxic to oligodendrocytes (Mason et al., 2000; Ma et al., 2011). The central part of the corpus callosum is known as a particularly sensitive region (Matsushima and Morell, 2001). To compensate for the death of oligodendrocytes, precursor cells differentiate into oligodendrocytes; however, these are also eliminated by cuprizone. For this reason, remyelination does not occur during cuprizone administration. When the administration is discontinued, oligodendrocytes differentiated from oligodendrocyte precursor cells can begin the remyelination process. Therefore, it is possible to experimentally produce a decrease in the amount of myelin due to ongoing demyelination and a subsequent decrease in the amount of myelin during the course of the remyelination process.

Previous studies have reported DTI results for this cuprizone model. Falangola et al. and Thiessen et al. compared cuprizone-treated to control mice to investigate the correlation between DTI and histological findings, but did not investigate the remyelination process (Thiessen et al., 2013; Falangola et al., 2014). Sun et al. examined a longer time course of DTI changes during demyelination and remyelination, but only compared these changes with the RD and AD values (Sun et al., 2006). In addition they did not correlate these values to histology (Sun et al., 2006). Guglielmetti et al. reported temporal changes in DTI parameters during demyelination and remyelination, but also did not compare to histological findings (Guglielmetti et al., 2016). Additionally, they found changes of the DTI parameters in the opposite direction during the demyelination process, compared with the previous three groups. Taken together, the results of these studies do not fully explain the temporal relationship between DTI parameters and histological information during demyelination and remyelination. To address this issue, we employed a reversible demyelination mouse model using cuprizone administration and investigated the time course of the DTI parameters' transition over the demyelination and remyelination processes in parallel with a histological evaluation of the myelination status as well as specific cellular subtypes such as oligodendrocyte precursor cells, microglia, and astrocytes. Finally, we examined the association of the DTI parameters with the myelin content during each process as well as with the cellular subtypes.

2. Materials & methods

2.1. Animal model

A total of 21 (7-week-old) male C57BL/6J mice were purchased from the Oriental Yeast Co., Ltd. (Tokyo Japan) The cuprizone treated groups (6 groups, n = 18) were fed a milled diet (CE-2, Clea, Japan) containing 0.2% cuprizone (Tokyo Chemical Industry, Tokyo, Japan) at 8 weeks of age. After 4 weeks of cuprizone treatment, a normal diet was resumed. At each time point (Fig. 1A), we sacrificed the corresponding group and collected the brains. The control group (0 week; n = 3, 8 weeks of age) was not treated with cuprizone. Animal experiments were approved by the Keio University Animal Experiment Committee in compliance with Keio University Animal Experimental Regulations (approval number: 12036-2). All animals were housed in temperature- and humidity-controlled rooms on a 12 h light/dark cycle (lights on at 8:00 a.m.).

2.2. DTI acquisition and analysis

An *ex vivo* DTI study of the mouse brains was performed with a 7 T Biospec 70/16 MRI scanner (Bruker Biospin GmbH; Ettlingen, Germany) equipped with actively shielded gradients at a maximum strength of 700 mT/m and a transmitting/receiving volume coil with an inner diameter of 22 mm. Under anesthesia (100 mg/kg ketamine and 10 mg/kg xylazine, i.p.), each animal was transcardially perfused with 10 ml of 0.1 M phosphate-buffer solution (PBS, pH 7.4) containing 4% (w/v) paraformaldehyde (PFA). The brains were post fixed overnight and then were immersed in 0.2 mM gadolinium containing PBS for one week. The brains were firmly fixed in an acrylic tube filled with Fluorinert (Sumitomo 3M Limited, Tokyo, Japan) to minimize the signal intensity attributable to the medium surrounding the specimen. A 3D DTI-Spin Echo protocol was used for the acquisition of the DTI images with the following parameters: time of repetition (TR) = 600 ms, time of echo (TE) = 20 ms, number of average = 1, b-value = 1000 and 2000 s/mm^2, number image with b = 0 s/mm^2 = 4, number of encoding direction of motion probing gradient (MPG) = 30, field of view = 19.2 × 12.8 × 9.6 mm, matrix size = 154 × 102 × 77, spatial resolution = 125 × 125 × 125 μm^3, acquisition time = 48 h.

The DTI analysis was performed using Diffusion Toolkit (Massachusetts General Hospital, Boston, Mass, http://trackvis.org/dtk/). A diffusion tensor was modeled at each voxel of the DTI image. Scalar anisotropy and diffusivity maps were obtained from the resulting diffusion tensor eigenvalues (λ_1, λ_2, λ_3) which captured the length of the longest, middle, and shortest axes of the ellipsoid. FA, MD, RA, and AD were calculated from the standard formula (Le Bihan et al., 2001; Alexander et al., 2007):

$$\mathrm{MD} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \tag{1}$$

$$FA = \sqrt{\frac{1}{2}} \frac{\sqrt{(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \tag{2}$$

$$\mathrm{RD} = \frac{\lambda_2 + \lambda_3}{2} \tag{3}$$

A region of interest (ROI) was manually set at the middle portion of the corpus callosum using the b0 image with MRIcro software (http://www.mricro.com/). The same ROI was used to measure FA, MD, and RD on the respective maps (Fig. 1D).

2.3. Histology

After the *ex vivo* DTI study, the fixed brains were cryoprotected in 20% sucrose/phosphate buffered saline (PBS) overnight, frozen, and cut at a 25 μm thickness on a cryostat. The resultant sections were used to evaluate the myelin content by immunohistochemistry (IHC) and the number of oligodendrocyte precursor cells, microglia, and astrocytes by in situ hybridization (ISH).

2.3.1. Immunohistochemistry

After antigen retrieval with a citric acid buffer (10 mM, pH 6.0) at 98 °C for 40 min, an anti-proteolipid protein (PLP) rat monoclonal antibody (AA3, 1:1 dilution, hybridoma supernatant, a gift from Dr. Kazuhiro Ikenaka at National Institute for Physiological Sciences, Japan (Inamura et al., 2012)) was applied overnight at room temperature (RT). Sections were then incubated with biotinylated anti-rat IgG secondary antibodies (1:250; Vector Laboratories) for 90 min at RT, an avidin-biotin complex (Elite ABC kit, Vector Laboratories) for 30 min at RT, and then the colorimetric

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