Assessing neuronal metabolism in MS by modelling imaging measures

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Introduction

The role of mitochondrial dysfunction in the pathogenesis of MS is becoming increasingly evident[1]. ^{1}H-MR spectroscopy (MRS) can provide a combined measure of both neuronal/axonal loss[2] and mitochondrial metabolism[3], in the quantity NAA (N-acetyl-aspartate). Isolating the mitochondrial metabolic component of NAA would greatly inform our understanding of disease mechanisms in the central nervous system. In the present study, we propose an indirect estimation of mitochondrial metabolism by modelling the NAA concentration with imaging measures that reflect structural axonal damage. The residual variance in NAA should then reflect mitochondrial metabolism. The imaging measures included in the model as measures of axonal loss were: (i) the DTI-derived axial diffusivity (AD), which is the principal eigenvalue of the diffusion-tensor, and is thought to reflect axonal integrity[4] in regions where fibres are highly aligned; and (ii) the cord cross-sectional area, which is reduced in association with neurodegeneration[5]. We performed this methodology in a group of patients with MS, studied six months after a spinal cord relapse, to assess the contribution of mitochondrial metabolism to neurological disability independent of measures of structural damage.

Methods

Subjects: Thirteen MS patients [mean age 36y.(SD8.02), 8 women, 12 RR and 1SP, median Expanded Disability Status Scale (EDSS) 3.5 (range1-6.5), mean timed 25-foot walk test (TWT) 7.3secs. (SD1.8), mean 9-Hole Peg Test (9HPT) 22.9 (SD3.2), mean MS Walking Scale-12 (MSWS-12) 32.5 (SD12.8)] underwent spinal cord MRS and diffusion-tensor imaging six months after a relapse, which was due to at least one lesion between C1 and C3 (**Fig.1A**). 13 age-matched healthy subjects (mean age: 40.8y.(SD12.6), 7 women) were studied.

MRI protocol and analysis at 1.5T: (1) T2 and PD-weighted imaging (TR3300ms/TE110ms and TR3000ms/TE8.9ms, echo train length 33 and 11, FOV=240x240mm^2, matrix=256x224, interpolated to 512x512, 12 contiguous sagittal-oblique (Fig.1A) and coronal-oblique slices, 3mm slice thickness). One patient, showing swelling of the cord at the level of the lesion, was excluded from the analysis. (2) MRS with the PROBE(PRESS) sequence (TE30ms, 192 averages) with CHESS water suppression, cardiac gating and spatial saturation pulses. A single voxel was placed on coronal and sagittal T2 images along the main axis of the cord between C1 and C3 (**Fig.1B**), as previously described[6]. The LCModel was used to calculate the concentration (in mM) and the %SD of the total NAA (**Fig.1B**). (3) CO-ZOOM-EPI[7] (TE96ms, cardiac gating, 6 b0, diffusion gradients applied along 60 optimized diffusion directions, maximum b factor=1000s/mm², FOV=70x47mm² reconstructed to 1x1mm², 30 contiguous axial slices, 5mm slice thickness). The diffusion tensor was estimated on a voxel-by-voxel basis with the RESTORE (for robust estimation of diffusion tensor with outlier rejection) algorithm, using Camino software (www.camino.org). Two patients' data-sets containing four volumes affected by artefacts were excluded from the analysis. Maps of AD were obtained. Four regions-of-interest (ROIs) were placed on the b0 images, between C1 and C3, in the white matter columns, where white matter tracts are located, and then automatically transferred to AD maps (**Fig.1C**). In particular, two ROIs of 4 voxels were placed in the posterior part of the lateral columns (where the lateral cortico-spinal tract is known to be located), and two ROIs of 6 voxels were placed in the anterior and posterior columns (**Fig.1D**). For each subject, the mean value of AD (and RD) of the four ROIs was calculated. (4) FSPGR (volume-acquired, inversion-prepared, fast-spoiled-gradient recalled) sequence (TR13.2ms, TE4.2ms, TI450ms, flip angle 20°, FOV 250x250mm², matrix 256x256, 60 contiguous sagittal slices, 1mm slice thickness). The cord cross-sectional cord area at C2–C3 was calculated using a semi-automated method[8] (**Fig.1E**).

Statistical analysis: The differences in imaging measures between patients and controls were assessed using the Mann-Whitney U test. We assumed that NAA concentration is explained by the combination of axonal structural integrity and mitochondrial metabolism. This relationship can be represented in *general linear form* as:

$$Y = X1 + X2 + \varepsilon$$

Y is the NAA concentration, X1 is the AD and X2 is the cord cross-sectional area. ε is the residual variance in NAA concentration (termed **ResNAA**) after accounting for the structural measures (i.e., AD and cord cross-sectional area), and therefore reflects neuronal mitochondrial metabolism. We applied this model to determine ResNAA for the MS patients. Next, to determine the association between mitochondrial metabolism and clinical disability, separate linear regression analyses were performed, in which each clinical score (9HPT, inv.TWT and MSWS-12) was regressed on ResNAA. For EDSS, an ordinal logistic regression was specifically run using three EDSS categories.
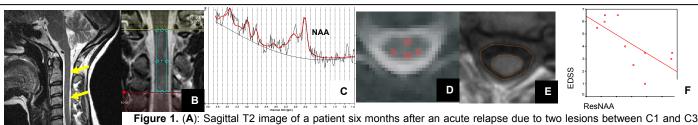
Results

Patients showed lower NAA levels and cord cross-sectional than controls [NAA: mean 4.2mM (SD 1.97) vs. 5.9 (1.05), p=0.034, cord area: mean 71.36mm^2 (SD 7.85) vs. 79.20 (6.16), p=0.007]. Patients with higher ResNAA were less likely to have a higher EDSS (odds ratio= 0.09, p= 0.034, 95%CIs 0.01, 0.84) (**Fig.1F**). Lower ResNAA was associated with worse walking ability (inv.TWT: p=0.024, coeff.= 0.026, 95%CIs 0.004, 0.05; MSWS-12: p= 0.013, coeff.= -10.7, 95%CIs -18.4, -2.9).

Discussion

This study proposes a method to explore *in-vivo* the contribution of neuronal mitochondrial metabolism to disability. Our method can be applied to other areas of the CNS, as long as the measures are calculated in the same region, and extended to other diseases, in which mitochondrial impairment is central to the pathogenesis. We found that a component of NAA, measured in the cervical cord, possibly reflecting mitochondrial metabolism, is significantly associated with clinical disability in MS patients after a cervical cord relapse. This is independent of measures of structural integrity, which do not correlate with disability, supporting the hypothesis that mitochondrial dysfunction plays an important role in contributing to neurological impairment in MS. Future brain longitudinal studies in MS should answer whether mitochondrial dysfunction is a major contributor to the progression of disability over time. On the other hand, mitochondrial dysfunction, as reflected in the NAA levels, can be partially reversible[9-10], and mitochondrial over-activity may reflect a compensatory repair mechanism which results in clinical recovery. Consequently, the clinical application of a method that permits the extraction of *in-vivo* mitochondrial metabolism by statistical modelling, is of obvious significance, and potentially may be extended to other disorders and key areas of the CNS.

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Figure 1. (**A**): Sagittal T2 image of a patient six months after an acute relapse due to two lesions between C1 and C3 (yellow arrows). (**B and C**): MRS volume of interest between C1 and C3 on the coronal image and corresponding spectrum. [NAA=3.94mM/l (%SD11)]. (**D**): Four regions-of-interest located in the posterior part of the lateral columns and in the anterior and posterior columns of the AD maps. (**E**): FSPRG image at C2-C3 with the regions-of-interest contoured to calculate the cord area[7]. (**F**) Scatter plot of ResNAA vs. EDSS (Rsq=0.5).