Multimodal MRI changes in cortical grey matter following formalin fixation

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INTRODUCTION: Post mortem multiple sclerosis (MS) brain is being used to establish the pathological correlates of changes detected using MRI (1). Formalin fixation of the brain tissue introduces a potential confounder that may affect the inference of in vivo changes from MR/histology studies. Effects of fixation on quantitative MR indices in MS white matter (WM) have been reported (2). This study investigated changes following fixation of quantitative MRI indices in MS cortical grey matter (CGM). We also determined the relative proportion of CGM lesions (CGML) and healthy looking cortex (HLC) on histological sections of the same cases and estimated the contribution of these two tissue features towards MR indices.

METHODS: Fifteen MS brain samples were studied unfixed 51 hours [standard deviation (SD) 28] post-mortem, and after 64 days [40] of fixation. Using a 1.5T GE scanner the following 2D datasets were acquired (1): (i) gradient echo (GRE) to calculate $T_1$, (ii) dual spin echo (SE) (with/out MT pulse) to map magnetisation transfer (MT) ratio (MTR), (iii) spoiled GRE to map the ‘fraction of macromolecular protons’ from quantitative MT ($f_B$) (for details see ref 2) and (iv) SE $T_2$ weighted ($T_2W$) images. Starting with the unfixed cases, on $T_2W$ SE images (fig 1) three regions of interest (ROI) were drawn in the cortical GM (fig 2), their mean and SD extracted and averaged. Regions of non-cortical brain tissue that potentially would have contaminated ‘pure’ CGM maps were removed (fig 3 & 4). Based on the three CGM ROI CGM masks were produced by thresholding the mean of the CGM ROI ± a multiple of their SD (fig 5). These masks were then applied to the quantitative MR maps of the each case to obtain an average ‘CGM’ map (fig 6). CGM maps for the fixed cases were acquired in the same way. Contrast between CGM and normal-appearing (NA) WM, and correlation between CGM and mean WM (NAWM + WM lesions)/2 values based on previously published data (2) were also investigated. Forty-eight histological sections stained for myelin basic protein from the above 15 MS cases were used to identify CGM and HLC. CGM was classified into four lesion types (Fig 7) (3). The proportion of HLC and CGM was obtained using Image Pro Plus mounted on a PC that was connected to a Leica Axiostop microscope. Student’s $t$ test and regression models were used for analysis.

RESULTS: Differences between unfixed and fixed CGM were detected for $T_1$ (1156ms [SD 216] vs 617ms [114], p<0.01), MTR (29.1pu [2.5] vs 24.1pu [3.3], p<0.01) and $f_B$ (3.2pu [2.3] vs 5.4pu [0.7], p<0.01). In 48 tissue blocks, 90 CGML (type 1: 16; type 2: 12; type 3: 59; type 4: 3) were detected. The proportion of CGML compared to HLC was highly variable ranging from zero to 33.8% (except for one outlier in which 59% of the cortex was demyelinated). On average, 18.6% of the total CGM was demyelinated. Contrast between CGM and NAWM was not reduced for all cases (3.2pu [2.3] vs 5.4pu [0.7], p<0.01). In 48 tissue blocks, 90 CGML (type 1: 16; type 2: 12; type 3: 59; type 4: 3) were detected. The proportion of CGML compared to HLC was highly variable ranging from zero to 33.8% (except for one outlier in which 59% of the cortex was demyelinated). On average, 18.6% of the total CGM was demyelinated. Contrast between CGM and NAWM was not reduced for all cases (3.2pu [2.3] vs 5.4pu [0.7], p<0.01). When comparing cases with high vs. low CGML load the proportion of CGML vs. HLC did not significantly affect the qMR measures, which may have been due to (i) an increase of $f_B$ in healthy looking cortex harbouring only ~10% of the amount of myelin present in the WM and (ii) less than 19% of the cortex in this study being demyelinated.

CONCLUSION Formalin fixation results in a substantial drop of $T_1$, less so of MTR, and an increase of $f_B$ in post mortem MS CGM, similar to changes observed in the WM (2). These changes are likely due to a combination of (i) direct formaldehyde effects (4) and (ii) intra- and intermolecular cross-linking of macromolecules (5). The proportion of CGML vs. HLC in this study is very similar to earlier reports (3). Comparing cases with high vs. low CGM lesion load the proportion of CGML vs. HLC did not significantly affect the qMR measures, which may have been due to (i) a healthy cortex harbouring only ~10% of the amount of myelin present in the WM and (ii) less than 19% of the cortex in this study being demyelinated.

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FIGURES Production of cortical grey matter maps

Graphs Changes of quantitative MRI indices following formalin fixation