Differences in the Brain of Irradiated Mice Investigated with White Matter Imaging Techniques

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Target Audience: Clinicians and scientists who are interested in the quantification of white matter abnormalities or the effect of paediatric cranial irradiation on brain development.

Introduction: MR imaging techniques are commonly used to assess white matter (WM) damage pre-clinically to study mechanisms controlling neurodegenerative diseases. Such techniques include magnetization transfer (MT), susceptibility weighted (SW) and diffusion tensor (DT) imaging. The use of each is interesting because they identify differences in macromolecular content [1], tissue susceptibility [2], and axonal diffusion properties [3], respectively, that can be used to make inferences about WM integrity. Pediatric cranial irradiation results in cognitive late effects, such as memory loss, that have been correlated with a reduction in WM volume detectable with conventional MRI [4]. The objective of this study was to assess the damage to WM in the irradiated mouse using the three MR methods described.

Methods: Animals: At 2.5 weeks (infant) female C57Bl/6J mice were irradiated with a single dose of 7 (irradiated, n=8) or 0.6 (control, n=7) Gy to the whole brain. All mice were sacrificed 3.5 weeks later (late adolescence) and *ex-vivo* MT, SW and DT images were acquired separately on a 7T scanner (Agilent Technologies Inc.). While all brain were scanned with MT and DTI, only 7 irradiated and 5 control were scanned with SW. **MT:** MT reference images were acquired using a 3D spin echo sequence (98µm isotropic resolution, 4 averages, TR/TE=300/8ms) followed by MT image acquisition using the same sequence and starting with an MT saturation pulse (Gaussian, +3500Hz off resonance, 20ms long, 5x per TR). Complete acquisition of both reference and MT images took ~ 14.5hrs. The magnetization transfer ratio (MTR) was calculated as the percent decrease in voxel intensity from the reference to the MT image, with a correction by the average MTR value in the cerebral cortex. **SW:** SW images were acquired using a 3D gradient echo sequence (61µm isotropic resolution, 2 averages, TR/TE=400/18ms). Phase processing was performed using the methods described by Liu et al [2]. Briefly, this consisted of 3D phase unwrapping, removal of the background phase with spherical mean value filtering, and calculation of the quantitative magnetic susceptibility (QMS) value with a correction by the average QMS value in the cerebral cortex. **DT:** DT images were acquired using a 3D diffusion weighted fast-spin-echo sequence (78µm isotropic resolution, 1 average, 6 echoes at 6ms echo spacing with an initial TE of 30ms, TR=350ms, ~14 h scan time). Five low (b=0 s/mm^2) and 30 high (b=1917s/mm^2, 30 directions) b-value images were acquired. Mean diffusivity (MD), fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD) were computed using the FSL software package (FMRIB, Oxford UK). **Image Analysis:** Each WM measurement was processed separately. For each, an automated registration process was used to align all reference images to a consensus average space. The computed deformations were applied to each corresponding measurement image. 62 automatically-segmented brain structures (22 WM structures) were aligned to each of the three consensus averages and mean MTR, QMS, MD, FA, AD and RD were calculated for each structure. Where required, p-values were corrected for multiple comparisons through the false discovery rate (FDR) method.

Results: A significant difference (5%FDR, unpaired t-test) in MTR and QMS between control and irradiated groups was identified in 20, and 11 of the 62 structures, respectively. A significant difference was not detected for any of the DT measurements (FDR>20%). Presented in **Figure 1** are coronal and horizontal slices of the average image for MTR, QMS, MD and FA. Structures found to be significant are highlighted in blue, where the colour bar shows the percent difference in MTR and QMS from the controls. For reference, two major WM tracts, the corpus callosum (CC) and anterior commissure (AC) have been identified. **Figure 2** shows a comparison of control and irradiated corpus callosum values (normalized to control values), for all measurements assessed.

Discussion: MT and SW imaging have been shown to be predominantly sensitive to demyelination [1, 2]. In contrast, many DTI measurements remain relatively unchanged in the presence of demyelination, and are instead readily altered by axonal damage or remodeling [3]. Radial diffusivity has also been shown to be predictive of myelin damage. Unfortunately, with the current model, it fails to demonstrate sensitivity to the differences detected by MT and SW imaging. Formaldehyde fixation has been shown to increase water permeability in tissue and affect DTI measurements [5]. As a result, differences in axonal and myelin integrity, detectable with DTI, may be overshadowed by the changes in tissue properties introduced with our fixation method.

Conclusion: Previous research has identified significant volume loss in many WM structures of the mouse brain due to irradiation in infancy [6]. The current study has demonstrated damage to the remaining WM that is detectable with MT and SW imaging. The hypothesis that these are sensitive to demyelination is consistent with previous work [6] that demonstrated a decrease in myelin basic protein staining (a myelin marker) following irradiation. Our future work will investigate the effect of cranial irradiation on a mouse strain protected from oligodendrocyte (myelin forming cell) death. The aforementioned MR imaging methods will be used to assess the pathological changes that result.

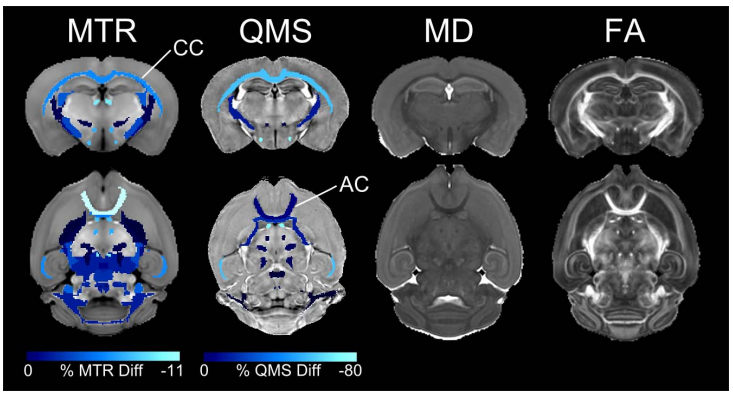

Figure 1. Coronal (top) and horizontal (bottom) slices of the average brain for each measurement collected. Structures highlighted show a percent difference, as indicated by the colour bar, between control and irradiated that survives multiple comparisons (1% FDR). CC=Corpus Callosum. AC = Anterior Commissure.

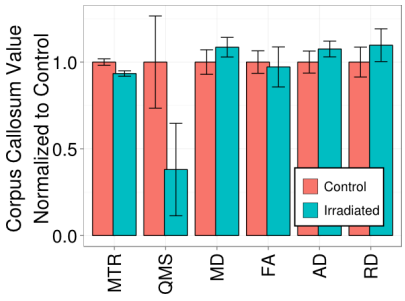

Figure 2. Normalized WM measurements in the corpus callosum. Each value is normalized to the average control result. Error bars are 95% CI.

References: [1] K Schmierer et al. (2004) Annals of Neurology **[2]** C Liu et al. (2011) NeuroImage **[3]** J Zhang et al. (2012) Trends Neurosci **[4]** RK Mulhern et al. (2003) Lancet Oncol. **[5]** TM Shepherd et al. (2009) Magn Reson Med **[6]** LM Gazdzinski et al. (2012) Int J Radiat Oncol Biol Phys