In vivo Chromium-enhanced MRI of Normal and Injured Retinas

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INTRODUCTION: The retina has a unique lipid profile with the highest level of long-chain polyunsaturated fatty acids in the body (1). Although disruption of lipid metabolism is known to play a key role in the onset and progression of a variety of retinal diseases (2), there is surprisingly little information regarding changes in global lipid profiles between normal and diseased retinal tissues (1). Chromium (Cr) has been used histologically to stabilize lipid fractions in the retina (3), and is suggested to enhance oxidizable lipids in brain MRI (4). This study explored the feasibility, sensitivity and specificity of in vivo chromium-enhanced MRI (CrMRI) of retinal lipids, by determining its spatiotemporal profiles and toxic effect after intravitreal Cr(VI) injection to normal and injured adult rats.

MATERIALS AND METHODS: Animal Preparation: Sprague-Dawley rats (N=39) were divided into 4 groups. In the Cr dose groups (Group 1, n=20), 4 normal rats from each dose group were intravitreally injected with 3 µL potassium dichromate solution into the left eyes at a concentration of 1, 5, 10, 50 or 100 mM under isoflurane anaesthesia. CrMRI was performed at 1 day post-injection. In the Cr time profile group (Group 2, n=6), 6 normal rats were injected with 10 mM of Cr(VI) at 3 µL to the left vitreous. CrMRI was performed before, and at 1 day, 1 week and 2 weeks post-injection. In Group 3, 7 normal rats were injected with Cr(VI) at 3 µL and 10 mM to the left vitreous. The animals were euthanized 1 day later and both eyes were enucleated and preserved. Four pairs of eyes were MRI scanned overnight. Oil red O lipid histology was performed to another 3 pairs of eyes to compare retinal lipid contents with CrMRI signals. In Group 4 (n=6), neonatal hypoxia-ischemia (HI) was induced to postnatal day 7 rats by left common carotid artery ligation followed by hypoxia at 8% O_2 and 36-37 ^oC for 2 hours. CrMRI was performed 12 months later, at 1 day after intravitreal Cr(VI) injection to both eyes at 3 µL and 10 mM. To evaluate the acute and chronic effects of Cr toxicity in vivo, $MnCl_2$ solution (3 µL, 50 mM) was intravitreally injected into both eyes of 3 rats from each Cr dose in Group 1 immediately after CrMRI at 1 day post Cr(VI) injection. $MnCl_2$ was also injected into both eyes of all rats in Group 2 after CrMRI at 2 weeks post Cr(VI) injection. Mn-enhanced MRI (MnMRI) was performed to the posterior brain at 1 day after Mn^{2+} injection.

MRI Protocol: All MRI measurements were acquired using the 7T Bruker scanner. For in vivo CrMRI and MnMRI, 2D RARE T1WI was acquired covering the center of both eyeballs, and the superior colliculi (SC) and lateral geniculate nuclei (LGN) respectively at TR/TE = 475/8.8 ms, spatial resolution = 125x125x800 µm^3 and total scan time = 10 mins. For ex vivo CrMRI, 3D FLASH T1WI was acquired at TR/TE = 26/4.5 ms, with an isotropic resolution of 50x50x50µm^3 and total scan time = 16 hrs 45 mins.

Data Analysis: The ratios of T1W signal intensities (SI) between left and right retinas of Group 1 after Cr(VI) but before Mn^{2+} injection were compared among Cr doses using one-way ANOVA tests with post-hoc Bonferroni's multiple comparison tests, while those in Group 2 were compared across time using repeated measures ANOVA tests with post-hoc Bonferroni's tests. The retinal profiles in high-resolution CrMRI and lipid histology were compared layer-specifically in Group 3. The T1W signal increases between left and right retina in Group 4, and the T1W SI between left and right SC or LGN within each Cr dose in Groups 1 and 2 after Mn^{2+} injection were compared using two-tailed paired t-tests. Data were presented as mean ± standard deviation. Results were considered significant when $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ns: not significant).

RESULTS AND DISCUSSIONS: One day after 3µL Cr(VI) administration at 1mM to 100mM, the normal retina exhibited a dose-dependent increase in T1W hyperintensity until 50mM (Fig. 1). Time-dependently, significant T1W hyperintensity persisted up to 2 weeks after 10mM Cr(VI) administration (Fig. 2). 3D-CrMRI of ex vivo chromated normal eyes at isotropic 50µm resolution showed at least 5 alternating bands across retinal layers, with the outermost layer being the brightest (green arrows, Fig. 3). This agreed with histology indicating alternating lipid contents with the highest level in the photoreceptor layer of the outer retina (Fig. 3)(5). In vivo CrMRI demonstrated reduced Cr enhancement in ipsilesional, HI-injured retina (Fig. 4), which might be partly due to lipid peroxidation or decreased cellular densities (6,7). While Cr(VI) reduction may induce oxidative stress and depolymerize microtubules (8), MnMRI after CrMRI showed a dose-dependent effect of acute Cr toxicity on Mn^{2+} uptake and axonal transport along the visual pathway (Fig. 5), with significantly reduced T1W SI in the right SC and LGN at Cr(VI) concentrations of 50 and 100mM ($p<0.05$) but not 1, 5 or 10mM ($p>0.05$). No significant change in MnMRI SI was observed between left and right SC or LGN at 2 weeks after 10mM Cr(VI) injection and 1 day after Mn^{2+} injection ($p>0.05$)(data not shown).

CONCLUSION: The results of this study demonstrated the feasibility, sensitivity and specificity of CrMRI for assessing the tissue properties in normal and injured retinas in vivo, and potentiated longitudinal CrMRI investigations of retinal lipid metabolism upon further optimization of Cr doses with visual cell viability.

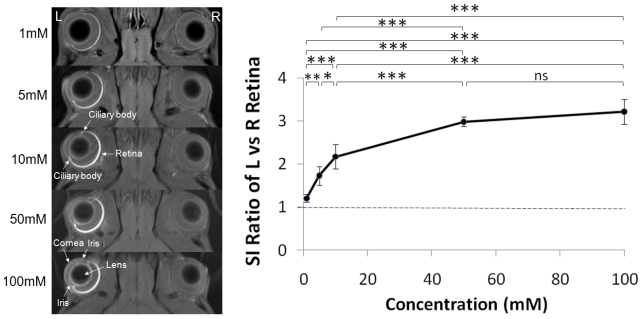

Fig. 1: In vivo T1W images (left) and T1W SI ratios (right) of normal adult retina at 1 day after intravitreal injection of 3µL potassium dichromate solution to the left eyes at 1, 5, 10, 50 and 100 mM.

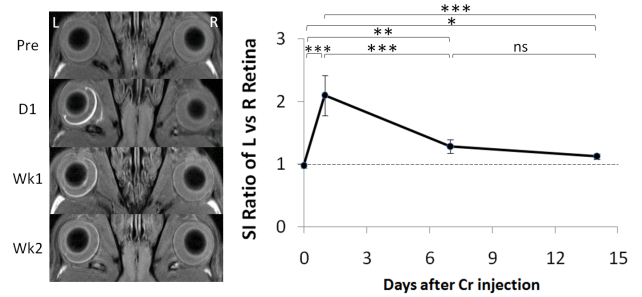

Fig. 2: In vivo T1W images (left) and T1W SI ratios (right) of the normal retina before (Pre, Day 0), and at 1 day (D1), 1 week (Wk1) and 2 weeks (Wk2) after intravitreal Cr(VI) injection into left eye at 3µL and 10mM.

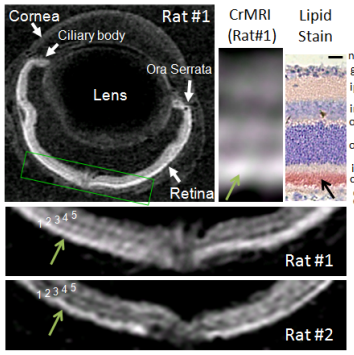

Fig. 3: Ex vivo 3D CrMRI of normal retina from two rats at 50x50x50 µm^3. Oil red O lipid stain (red) of retina counterstained with hematoxylin (blue) for cell nuclei showed the highest lipid contents in the photoreceptor layer (black arrow). Scale bar = 25 µm.

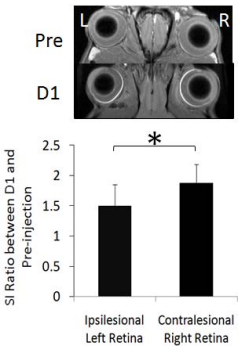

Fig. 4: In vivo CrMRI of HI-injured retina 12 months after left common carotid artery ligation and 2 hours of 8% O_2 at postnatal day 7.

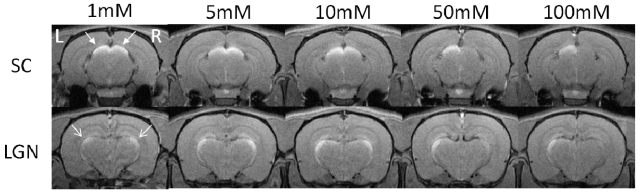

Fig. 5: Dose-dependent effect of Cr toxicity on Mn^{2+} uptake and transport along the visual pathway at 2 days after 3 µL Cr(VI) injection into left eye at 1mM to 100mM, and 1 day after $MnCl_2$ injection into both eyes.

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