Detection of Radiation-induced Brain Injuries by Multi-modal Magnetic Resonance Imaging

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INTRODUCTION: Radiation therapy plays an important role in the treatment of brain tumors, arteriovenous malformations and functional disorders such as epilepsy [1, 2]. However, *in vivo* studies examining the long-term effect in experimental radiosurgical models are not fully explored [3, 4]. While conventional MRI describes the cortical surface, deep gray matter and cerebrospinal fluid space anatomy, it does not provide insight into the complex organization of the cerebral white matter, nor does it provide functional or metabolic information [5]. This study aimed to demonstrate the integration of complementary structural, microstructural, metabolic and biochemical data using *in vivo* multi-modal MR imaging so as to better understand the late radiation damage in the rat brain in correlation to histopathological changes.

MATERIALS AND METHODS: Animal Preparation: The right hemibrains of Sprague-Dawley male rats (250-300 g, N=7) were irradiated with a single highly collimated 6 MV photon beam produced from a linear accelerator (Clinac 23EX, Varian Medical Systems Inc, Palo Alto, USA) at a dose of 25-30 Gy under fentanyl/fluanisone anaesthesia (0.4 ml/kg). The machine isocenter was located in the frontoparietal region. 12 months after radiation treatment, T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), proton magnetic resonance spectroscopy (^{1}H-MRS) and diffusion tensor imaging (DTI) were performed on all animals, while one of the rats underwent Mn-enahnced MRI (MEMRI) at 1, 2 and 5 days after intraperitoneal injection of $MnCl_2$ solution (3 ml/kg, 120 mM). Throughout the experiments, the left hemibrain did not expose to radiation and was served as an internal control. **MRI Protocols:** All MRI measurements were acquired utilizing a 7 T Bruker scanner. Under inhaled isoflurane anaesthesia (3% induction and 1.5% maintenance), animals were kept warm under circulating water at 37 ^{o}C and were imaged using a 38 mm rat brain quadrature resonator. 2D T1-weighted RARE sequence was acquired with FOV = 3.2 x 3.2 cm^2, matrix resolution = 256 x 256, slice thickness = 1 mm, number of slices = 10, TR/TE = 400/7.5 ms, RARE factor = 4 and NEX = 16; T2WI was performed under the same dimensions with TR/TE = 4200/38.7ms, RARE factor = 8 and NEX = 2; After shimming with FASTMAP, ^{1}H-MRS was performed using a PRESS sequence with TR/TE = 2000/20 ms and NEX = 128. A 4x4x4 mm^3 voxel was placed over the isocenter of irradiation, and another contralateral to the isocenter. Metabolite ratios were calculated using Sub-QUEST method in jMRUI [6]; SE-EPI diffusion weighted images were acquired with FOV = 3.0 x 3.0 cm^2, matrix resolution = 128 x 128, TR/TE = 3000/28 ms, NEX = 4, b = 0 and 1000 s/mm^2, number of shots = 4 and 30 diffusion directions. The diffusion tensors were extracted and diagonalized using an in-house Matlab program interfaced to the software for CNLS estimation for DTI (by Dr. CG Koay, STBB/LIMB/NICHD, NIH). **Histology:** After MR examinations, the rats were transcardially perfused with 4% paraformaldehyde. The brains were then removed, cut into 10 μm sections, stained with hematoxylin and eosin (H&E) to detect general morphological abnormalities, and immunostained for glial fibrillary acidic protein (GFAP), manganese superoxide dismutase (MnSOD) and glutamine synthetase (GS), which are markers for gliosis, oxidative stress and glutamate excitotoxicity, respectively.

RESULTS AND DISCUSSION: T2WI&DTI: The morphological structures of the ipsilateral corpus callosum and external capsule appeared to have lost their contrast to the surrounding gray matters in T2WI (4/7), whereby hyperintensity was found in the corresponding locations and in the hippocampus of trace map (3/7) as in Figure 1. An enlarged lateral ventricle represented by hyperintense signals in T2WI and trace map was also observed on the ipsilateral side in Figure 3 (4/7), which was in good correlation to our histopathological findings and the literature [3]; The fractional anisotropy at the fimbria of hippocampus has also dropped significantly (ipsi. vs contra. = 0.543±0.194 vs 0.827±0.044, $p<0.01$ in paired t-test), suggestive of degeneration in this sensitive white matter structure [1,7,8]. **MRS:** In Figure 2, significant increase in Cho:Cr and Lac:Cr ratios ($p<0.05$) was found in the voxel covering the isocenter of radiation, which partially included the hippocampus, its fimbria and the internal capsule. Together with the increase in trace value surrounding the hypointense radiation core (6/7), these indicated the presence of oedema [2]. The increase in Cho:Cr might also reflect demyelination in the white matter structures concerned [7]; There was a marginally significant increase in Glu:Cr ratio ($p=0.052$). We speculated this represented the higher intensity of GS in Figure 3, which was an astrocyte marker known to remove the potentially toxic excess of glutamate to accumulate as the relatively harmless product glutamine [9]. **MEMRI:** The pattern of Mn^{2+} enhancement at the site of radiation appeared to colocalize closely with GFAP, MnSOD and GS staining. Previous studies have shown that increased GFAP-positive fibers in the irradiated brain were accompanied by gliosis in the affected areas [10]. Astroglial cells highly immunostained for GFAP would also secrete reactive oxygen species, causing an upregulation of MnSOD and GS [11, 12], which are Mn dependent enzymes. It is possible that exogenous Mn^{2+} injection could lead to enhanced MEMRI detection of oxidative stress and gliosis [12, 13].

CONCLUSION: Multi-modal MR imaging potentially provides an *in vivo* assessment on the structural, microstructual, metabolic and biochemical aspects of radiation-induced brain damage in the same animal globally and non-invasively, and can be valuable in monitoring the pathophysiological cascades during the course of radiation-induced injuries.

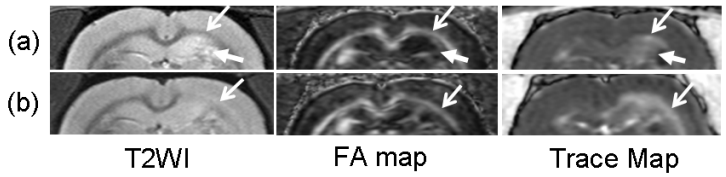

Fig 1: Representative MR images of 2 rats which showed an apparent loss of T2 contrast, reduced FA value and increased trace value at the ipsilateral corpus callosum and external capsule (open arrows), and an increase in T2WI signals and trace values in the ipsilateral hippocampus (solid arrows).

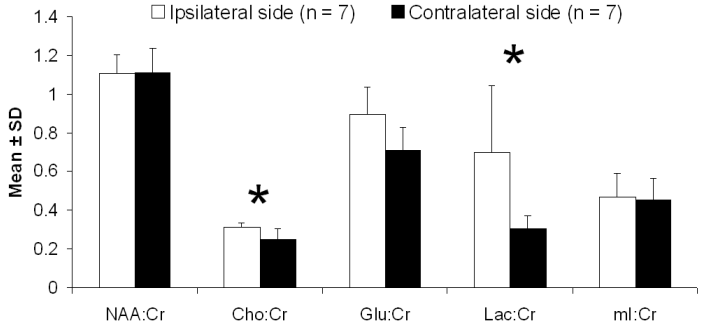

Fig 2: Metabolite ratios ipsilateral and contralateral to radiation isocenter 12 months after radiation treatment. * paired t-test between contralateral sides with $p < 0.05$.

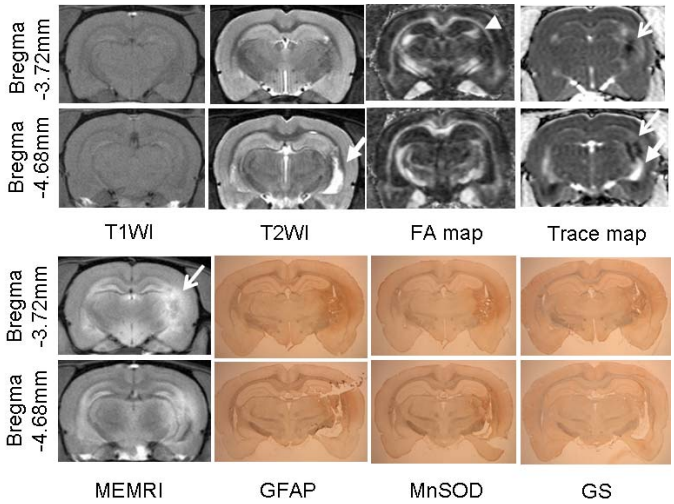

Fig 3: (Top) Typical multi-modal MR images without Mn^{2+} injection at bregma -3.72 mm and -4.68 mm, indicating an enlargement in the ipsilateral lateral ventricle in T2WI and trace map (solid arrows), reduced FA in fimbria of hippocampus (arrowhead), and an increase in trace value surrounding the hypointense radiation core (open arrows). (Bottom) Mn-enhanced MRI 24 hours after IP injection, with the corresponding colocalization of Mn-enhancement with GFAP, MnSOD and GS staining (open arrow). An enlarged lateral ventricle was also observed in the histological sections in correlation to that in T2WI and trace map.

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