A multiparametric MR approach for comparative assessment of neurometabolites and brain microstructural changes in mice model for cranial and whole body radiation exposure

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Introduction: The pathophysiology of radiation injury to CNS is not fully understood. It may be varied with the type of exposure (partial or whole body), radiation dose, size of radiation field etc. It is presumed that brain will respond differentially following cranial radiation and whole body radiation of same dose. In case of whole body radiation exposure, understanding the changes at early stages is more important as these changes might be helpful in development of markers for radiation injury to CNS during radiation accident. Moreover, to identify markers for whole body radiation accident, studies are required to compare changes following whole body radiation and focal radiation exposure. Diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (^{1}H MRS) are powerful technique for characterising the microstructural and metabolic responses in brain during abnormalities. We hypothesize in this study that DTI and MRS together will help us in elucidating the early differential response of brain following whole body or cranial irradiation in mouse model.

Aim of the study: To explore early microstructural and metabolic alterations in brain in mice after exposure to either cranial or whole body irradiation using DTI and MRS.

Materials and Methods: A total of 30 strain 'A' male mice (8 to 10 weeks old) were taken and randomly divided into three groups with 10 animals in each group. Mice were exposed to a radiation dose of 8 Gy for both cranial (single dose, Cranium) and whole body radiation group through Tele ^{60}Co irradiation facility unit. Controls (n = 10) were sham radiated. Brain MR experiments were performed at 0h, day 1, day 3, day 5 and day 10 post irradiation. Experiments were performed on a 7T system (Bruker Biospin Ettlingen, Germany) with 72-mm inner diameter (ID) linear birdcage coil as transmit coil and phase array coil (4 channel) as receive only coil. DTI images were acquired using a multi-slice, multiple-shot spin echo EPI sequence with the following parameters: repetition time (TR) / echo time (TE) = 5000 ms/34.46 ms, number of gradient encoding directions = 81, and b= 672 s mm-2. Java based DTI analysis software was used for the generation of FA (Fractional Anisotropy) and MD (Mean Diffusivity) maps. Regions of interest (ROI)s were placed on somato-sensory cortex (SMC), corpus callosum (CC), hippocampus (Hip), thalamus (Th) and hypothalamus (HTH) (Figure 1). For MRS, voxel was localised in the cortex-hippocampus region of mouse brain (1.5 x 3.5 x 3.0 mm^3; 15.75µl) and MR spectra were acquired using PRESS (Point Resolved Spectroscopy) sequence with TR of 2500 msec and TE of 20 msec and 512 averages. MRS raw data (FID) was processed using LC model for quantitation. FA and MD values from right and left hemisphere were pooled together for statistical analysis. One-way analysis of variance (ANOVA) with multiple comparisons using Bonferroni, Post Hoc test was performed to evaluate the differences in DTI and MRS measures among different time points.

Results and Discussion: MRI revealed no lesions or any obvious differences of image intensities in the T1 and T2 weighted images in any of the mice brain of all three groups. Out of both the DTI parameters measured in this study, most notable differences were observed in FA values in most of the brain regions in both the irradiated groups compared to controls. In hippocampus, thalamic and hypothalamic regions, there was consistent decrease in FA value in both the irradiated groups compared to pre dose values (Table 1). However, change in FA was initiated since day 1 and day 3 post irradiation in whole body irradiated group and cranial irradiated group respectively. Additionally in animals with whole body radiation exposure, SM and CC also showed decreased FA decreased since day 3 post-irradiation. Reduced FA in irradiated mice compared to controls could be because of reactive astrogliosis as supported by few earlier reports (1, 2). Additionally, neuroinflammatory processes including macrophage and microglia proliferation could also be the contributory factor for decreased FA. Reduced FA in our study could be a combined effect of reactive astrogliosis and neuroinflammatory response. MRS data showed creatine plus phosphocreatine (tCr), glycerophosphocholine plus phosphocholine (tCho), N-acetyl aspartate (NAA), total glutamine plus glutamate (Glx), myoinositol (mI) and taurine (tau) as the detectable and quantifiable metabolites in hippocampus. Significant changes were observed only in mI/tCr and tau/tCr ratios at day 3 post-irradiation in animals irradiated with whole body radiation dose compared to controls and whole brain irradiated group and continued to decrease till day 10 (Figure 2). Both tau and mI are one of the many organic osmolytes that are regulated in the brain and are believed to be located primarily in glia and absent in the neurons (3). The reductions of tau and mI found in this study may reflect hypo-osmolarity at the early stage of radiation injury. It could be interpreted that change in tau and mI levels in whole body irradiated group in this study might be a result of radiation induced neuroinflammation and osmotic disturbances. This further signifies that radiation induced inflammatory response may lead to metabolic and microstructural changes in the hippocampus region of the brain.

The differential response of brain at microstructural and metabolic level following cranial and whole body radiation exposure could be explained because of involvement of systemic inflammatory response of central nervous system in case of whole body irradiation only. During whole body irradiation, brain and body immune system as well as anti oxidant system is compromised because of free radicals generation after radiation exposure. In case of localised irradiation (as cranial irradiation in our study), radiation induced cellular response is limited to brain; giving an opportunity to antioxidant system of body to cope up with oxidative stress in localised damaged tissue.

Conclusion: This study for the first time documented the comparative account of microstructural and metabolic aspects of whole body and cranial radiation induced early brain injury using *in vivo* multiparametric MRI. These differential MRI findings can be valuable in monitoring the pathophysiological cascades in a single setting during the course of radiation induced early injury either due to accidental or intentional exposure to ionizing radiation.

Radiation type and day	Sensory Motor Cortex	Corpus Callosum	Hippocampus	Thalamus	Hypothalamus
Controls					
Day 0	0.25±0.04	0.53±0.02	0.16±0.02	0.25±.04	0.23±.04
Day 1	0.24±0.03	0.52±0.02	0.16±0.02	0.24±.02	0.23±.03
Day 3	0.24±0.03	0.49±0.01	0.15±0.02	0.25±.02	0.24±.02
Day 5	0.23±0.02	0.54±0.02	0.16±0.01	0.24±.02	0.23±.02
Day 10	0.24±0.02	0.52±0.02	0.15±0.01	0.24±.01	0.24±.02
Cranial Radiation					
Day 0	0.25±0.03	0.53±0.01	0.16±0.01	0.25±.02	0.22±.03
Day 1	0.21±0.02	0.51±0.01	0.12±0.01*	0.14±.02*	0.13±.02*
Day 3	0.24±0.03	0.48±0.04	0.10±0.01*	0.15±.02*	0.13±.03*
Day 5	0.23±0.03	0.54±0.02	0.09±0.02*	0.14±.06*	0.11±.02*
Day 10	0.22±0.03	0.50±0.07	0.11±0.02*	0.17±.05*	0.14±.03*
Whole Body Radiation					
Day 0	0.24±0.04	0.53±0.03	0.15±0.01	0.25±.02	0.23±.01
Day 1	0.21±0.02	0.47±0.04	0.15±0.02	0.23±.03	0.20±.04
Day 3	0.20±0.03*	0.43±0.06*	0.11±0.02*	0.20±.05	0.18±.07*
Day 5	0.17±0.02*	0.38±0.03*	0.10±0.02*	0.18±.03*	0.15±.03*

Table 1: Regional FA values at different time points post irradiation in irradiated and control groups

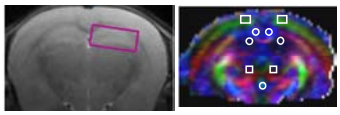

Figure 1: T_2 image and color coded FA map showing voxel and ROI position for MRS and DTI studies

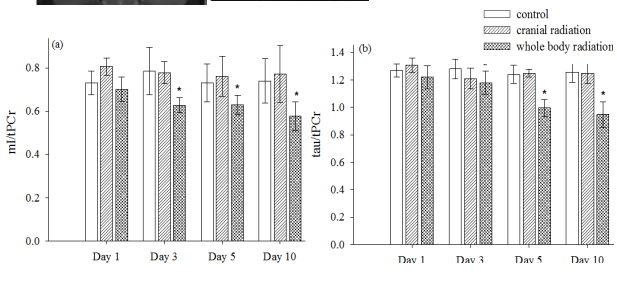

Figure 2: Temporal changes in mI/Cr+PCr and tau/Cr+PCr ratios in irradiated group compared to controls.

References: 1. Yang et al (2000) Neurosurgery, 47 : 407-415. **2.** Li et al (2003). Cancer Res, 63 :5950-5956. **3.** Brand et al (1993) Dev. Neurosci 15, 289-298.