Whole body Radiation Induced Early Delayed Changes in Mice Brain: Behavioral Evaluation and 1H MRS Study

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Target Audience: Researchers, Clinicians and Students.

Introduction: In the context of radiation syndrome following whole body radiation exposure, it was earlier assumed that the adult nervous tissue is radioresistant and is affected only at a high dose of 20-30 Gy. In last decade, it has now been understood that even low dose of ionizing radiation exposure influences central nervous system (CNS) functions and behaviour . There are several 1H MR spectroscopy based studies available in the literature that report radiation induced early and late delayed metabolic impairment in brain but these studies are limited to focal whole brain irradiation or targeted fractionated brain irradiation. Recent studies from our group has shown whole body radiation induced early microstructural and metabolic changes in brain using DTI and MRS techniques [1, 2]. However, effect of whole body radiation exposure on brain metabolism has still not been studied at early and late delayed phase. To extend our previous studies, the present study was designed to observe early delayed effects of radiation in hippocampus post whole body irradiation, behavioral alterations and metabolic impairment if any.

Objective: To explore the early delayed effects of 5Gy whole body irradiation on mice brain through Behavior evaluation and 1H MRS.

Material and methods: A total of 20 C57 male mice (8 to 10 weeks old) were taken and acclimatized for 48 hours in polypropylene cages under standard temperature, humidity conditions prior to group allocation and treatment. Out of 20 animals 10 animals were given 5Gy whole body radiation through Tele ^{60}Co irradiation facility (Bhabhatron II) with source operating at 2.496 Gy/min. The remaining 10 animals served as sham irradiated controls. The Behavioral and MRS experiments were carried out on 10 animals each at 3 months post irradiation. All animal handling and experimental protocols were performed in strict accordance by institutional animal ethical committee. For behavioral experiments all the mice were acclimatized in the behavior rooms 1 hrs prior to the start of the behavior experiment. The behavioral testing was performed during the light cycle from 10 am-2 pm by the same experimenter who had been handling the animals throughout. The spontaneous behavior activity in mice was evaluated using Opto-varimex 4 system. The working memory function was evaluated, the next day, using the novel object recognition test. All MRS experiments were performed on anaesthetised animals (i.p., xylazine (10mg/kg BW) and ketamine (80mg/kg BW)) at 7T on a Bruker Biospec system. The MRS voxel was localised in the hippocampus region of mouse brain (1.5 x 3.5 x 3.0 mm^3; 15.75µl). After local field homogeneity optimisation (FASTMAP) and water suppression (VAPOR), MR spectra were acquired using PRESS (Point Resolved Spectroscopy) sequence with TR of 2500 msec and TE of 20 msec and 512 averages. Total acquisition time of 21.33 min. was used for acquisition of MR spectra. MRS raw data (FID) was processed using LC model for quantitation. Independent Students t test was performed to evaluate the differences in metabolites among different time points.

Result and Discussion: The data showed impaired cognitive functions and altered metabolite levels during late acute phase of whole body radiation induced injury. In behavioural experiments, there was a significant impairment in the cognitive as well as exploratory functions at 3 months post irradiation in irradiated group as compared to controls (Figure 1). Quantitative analysis of 1H MRS using LCModel reported GABA, glutamine, glutamate, N acetyl aspartate (NAA), glutamine + glutamate (glx), taurine (tau), myo-inositol (mI) and choline + glycerophosphocholine (tCh) metabolites after normalisation with total creatinine in irradiated groups compared to controls (Figure 2). The results explained changes in mI and glutamine levels in irradiated animals compared to controls (Figure 3). Ionizing irradiation not only results in the acute generation of short-lived Reactive Oxygen Species, it also results in a persistent state of oxidative stress that extends up to several months or even years after irradiation. Moreover, hippocampal neurogenesis is important for hippocampal-dependent functions of learning and memory and the process is exquisitely sensitive to suppression by various stressors, including radiation and oxidative stress [3]. The decline in cognitive function at 3 months post irradiation could be due to radiation induced persistent oxidative stress. mI, a sugar like molecule, is considered as a glial marker and an increase in its content is believed to represent glial proliferation. Additionally, it also functions as an osmolyte, and its concentration is altered in many brain disorders including MCI and Alzheimer's disease [4]. Few other studies on humans have reported association of higher mI with reduced cognitive abilities [5, 6]. In our study, increased mI and decreased cognitive function at 3 months post whole body radiation exposure reflect that whole body radiation exposure may have long lasting effect on the cognitive performance. Recently, glutamine has been reported to have a neuroprotective effect against DNA Damage, Beta-Amyloid and H_2O_2-Induced Stress and glutamine levels have found to be reduced in Alzheimer's disease [7]. Reduced glutamine levels in our study might be associated for reduced cognitive functions as well. However, further studies regarding white matter and microstructural changes and correlative studies are further required to understand the mechanism behind the whole body radiation induced cognitive deficit.

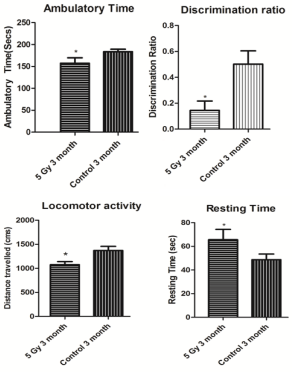

Figure 1: Bars showing the (a) Ambulatory Time (b) Discrimination ratio and (c) Locomotory activity and (d) Resting Time

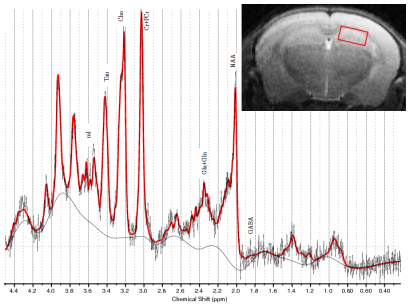

Figure 2: Representative LC Model processed ^{1}H MR spectrum from hippocampus region of mice brain

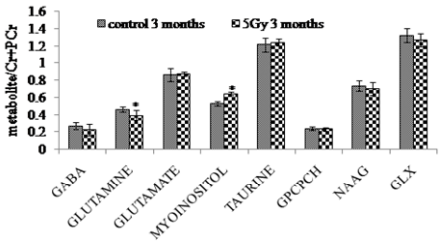

Figure 3: Bars showing metabolites changes in hippocampus at 3 months.

Conclusion: In the present study Behavior and MRS studies together were able to elucidate early delayed changes due to 5Gy whole body radiation in brain at cognitive and metabolic level. These findings can be valuable in evaluating the radiation induced persistent oxidative stress thus leading to cognitive dysfunction and metabolic impairment.

References: 1. Trivedi R, Khan AR, Rana P, et al. Radiation induced early changes in the brain and behavior: serial diffusion tensor imaging and behavioral evaluation after graded dose of radiation. J. Neurosci. Res. 2012; 90: 2009–2019. **2.** Rana P, Khan AR, Modi S, et al. Altered brain metabolism after whole body irradiation in mice: a preliminary in vivo 1H MRS study. Int. J. Radiat. Biol. 2013; 89: 212–218. **3.** Zaou Y, Corniolaa R, Leua D, et.al. Extracellular superoxide dismutase is important for hippocampal neurogenesis and preservation of cognitive functions after irradiation. PNAS. 2012; 109: 21522-527. **4**. Brand A, Richter-Landsberg C, Leibfritz D. Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. Dev Neurosci. 1993; 15:289–298 **5.** Siger M, Schuff N, Zhu X, et.al. Regional myo-inositol concentration in mild cognitive impairment using 1H magnetic resonance spectroscopic imaging. Alzheimer Dis Assoc Disord. 2009; 23(1):57-62. **6.** Beacher F, Simmons A, Daly E, et.al. Hippocampal myo-inositol and cognitive ability in adults with Down syndrome: an in vivo proton magnetic resonance spectroscopy study. Arch Gen Psychiatry. 2005 ;62(12):1360-5. **7.** Chen J, Herrup K. Glutamine Acts as a Neuroprotectant against DNA Damage, Beta-Amyloid and H_2O_2-Induced Stress. PLoS ONE.2012; 7(3): e33177. doi:10.1371/journal.pone.0033177.