DTI and Molecular Expression Based Studies Detects Radiation Induced Early Acute Neuroinflammatory Changes in Hippocampus

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Target Audience: Researchers, Clinicians and Students

Introduction: In last decade, it has been understood that ionizing radiation exposure influences central nervous system (CNS) functions and behaviour. Our recent studies have observed metabolic and microstructural changes in brain within 10 days post irradiation. However, changes in CNS are speculated due to systemic inflammatory response which can be observed within few hours of radiation exposure. The effect of enhanced systemic inflammatory response may further be observed as local neuroimmune and inflammatory response in CNS. However, CNS response has been poorly characterised immediately after radiation exposure i.e. within 24 hrs of exposure. There might be possibility of early acute effect on CNS as a consequence of systemic inflammatory response. The early acute effects could be studied using some sensitive techniques and DTI is one of the sensitive non invasive MR techniques that hold potential in identification of changes in brain at microstructural level by measuring the diffusion property of water molecules.

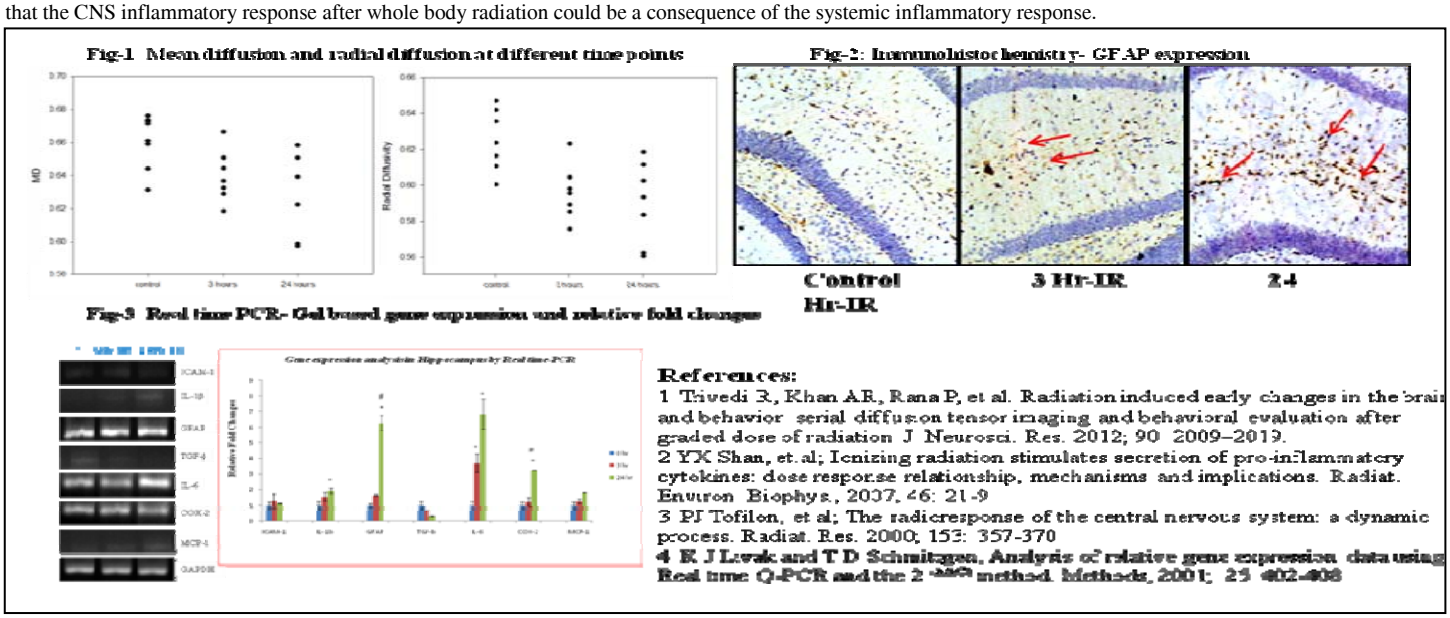
Aim of the study: The present study has been planned to look for immediate or early acute changes (within 24 hrs) in hippocampus using DTI technique and to link physiological changes with underlying molecular mechanism, RNA expression analysis has also been conducted on hippocampal tissue.

Materials and Methods: A total of 21 strain 'A' male mice (8 to 10 weeks old) were taken and randomly divided into two groups of which fourteen animals in the first group were exposed to single dose of 5 Gy whole body radiation through Tele ^{60}Co irradiation facility unit, remaining seven animals served as sham irradiated controls. Brain MR Experiments were performed at 3 and 24 hrs 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 from hippocampus. FA and MD values from right and left hemisphere were pooled together for statistical analysis. For mRNA expression of inflammatory markers, animals (n = 7 at each time point) were sacrificed at 3 and 24 hrs post irradiation. Controls were also sacrificed following MR experiments and hippocampus was immediately dissected out and placed in liquid nitrogen. mRNA was isolated by trizol method (260/280nm= >1.9) and real time rt-PCR was carried out by using different primers like ICAM-1, IL-1β, GFAP, TGF-β, IL-6, COX-2, MCP-1 and GAPDH. The relative fold changes were calculated by using $2^{-\Delta\Delta Ct}$. The statistical analysis in DTI and gene expression was carried by one-way analysis of variance (ANOVA) with multiple comparisons using Bonferroni, Post Hoc test to evaluate the differences in DTI and gene expression measures among different time points. Immunohistochemistry was carried out against GFAP expression at both the time points, in which DAB was used as chromogen and counter stain with haematoxylin.

Results: The average FA, MD, AD and RD values were collected from the ROIs placed in hippocampus in both the groups. Out of all the DTI parameters measured in this study, most notable differences were observed in MD and RD in irradiated groups compared to controls. The results showed marginal decrease in mean diffusivity at 3 h post irradiation but significantly decreased level at 24 hours post irradiation. No significant change in FA values was observed at any time point. Marked changes in radial diffusivity were further detected compared to axial diffusivity (Fig-1). The GFAP expression was marginally increased at 3 hr and significantly increased at 24 hrs as confirmed by immunohistochemistry (Fig-2). Neuroinflammatory response in terms of mRNA expression of IL-1β and IL-6 were significantly increased, whereas anti-inflammatory TGF-β was decreased at 3 as well as 24 hrs post irradiation as compare to control. Other genes like GFAP, COX-2, ICAM-1 were marginally increased in relative fold changes as compare to control (fig-3). However, increased expression was more prominent at 24hrs post irradiation time point.

Discussion: Decreased MD, RD as early as 3 hrs in hippocampus immediately following irradiation indicated cytotoxic edema and inflammatory response to the injury. Altered diffusivity depicts change in diffusion characteristics of the intracellular and extracellular water compartments including restricted water diffusion. The restricted water diffusion could be due to infiltration of inflammatory markers in the brain across the blood brain barrier due to elicited systemic inflammatory response. The neuroinflammatory response is further supported by increased mRNA expression of cytokines (IL-6, IL-1β) in hippocampus. Increased expression of IL-1β in our study is in line with earlier studies showing increased expression and induction of cytokines in CNS post whole body radiation. Enhanced neuroinflammatory response is further supported by low expression of anti-inflammatory TGF-1β post irradiation. Besides neuroinflammatory response, increased gliosis could be another reason for restricted water diffusion as increased expression of GFAP as well immunohistochemistry staining strongly support astrogliosis. Therefore, it is suggested that CNS particularly hippocampus response post irradiation occur even during early acute phase.

Conclusion: The study revealed altered DTI parameters MD and RD in particular in irradiated animals compared to controls during early acute phase i.e. within 24 hours of radiation exposure. The DTI changes along with enhanced cytokines expression in hippocampus depicted neuroinflammatory response and it is conceivable that the CNS inflammatory response after whole body radiation could be a consequence of the systemic inflammatory response.



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