Differential Effects of Fractionated and Single Radiation Dose on Diffusion Tensor Imaging in Mice Brain

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Introduction: Radiation therapy is an effective cancer therapy. Studies from exposed human and animals indicate that ionizing radiation can affect a wide variety of tissues particularly those with greater levels of cellular turnover and divisions. It has been argued that central nervous system (CNS) has a limited repertoire of responses to radiation injury but sometimes it kills healthy brain tissue as well - a severe side effect called radiation necrosis. Necrosis (a late effect of high doses of radiation) can cause headaches, seizures, or even death in a small number of cases (1). To minimize this radiation injury a non-invasive radiation therapy can be given repetitively over several weeks to months, minimizing side effects for uninvolved normal tissues. This repetitive treatment is called fractionation because a small fraction of the total dose is given in each treatment. It is a fact that even at very small dose levels, the central nervous system may be involved in some way without being observably damaged. Magnetic resonance imaging has proven to be a very sensitive technique to detect axonal demyelinization and vasogenic brain edema caused by capillary endothelial damage. But most studies have shown that conventional MR imaging is of limited benefit, because the sequences are not sensitive enough to depict early injury after irradiation (2). However, MR imaging methods, like diffusion tensor imaging (DTI), allow an in vivo examination of the microstructure of the brain tissue (3). The aim of our study was to investigate the role of quantitative DTI in defining the microstructural damage in mice brain after fractionated and single whole brain sub-lethal dose irradiation by using DTI technique.

Materials and methods: Male strain 'A' mice of age 8 to 10 weeks old (n=15) were taken and acclimatized for 48 hours in polypropylene cages under standard temperature, humidity conditions prior to group allocation and treatment. Out of 15 animals 10 animals were given 8gy cranial irradiation i.e. five animals were exposed to a singe dose of 8gy and five were exposed to fractionated dose of 8 gy i.e 2Gy at alternate days. The remaining 5 animals served as sham irradiated controls. All animals were anesthetized using mixture of ketamine and xylazine. Mice were exposed to a radiation dose through Tele 60Co irradiation facility unit gamma irradiation facility (Bhabhatron II) with source operating at 2.29 Gy/min. Brain MRI experiments were performed at 0h, day 1, day 3 and day 5 post 8gy irradiation. All MR imaging was performed in a Bruker Biospin 7.0 Tesla 30 cm horizontal bore magnet (Bruker Biospin Ettlingen, Germany) with resonant frequency of 300 MHz. Signal excitation was accomplished with a 72-mm inner diameter (ID) linear birdcage coil, and signal reception was achieved using phase array coil for mouse head. MRI protocol included high-resolution anatomical RARE images and DTI images. 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. The other parameters were: acquisition matrix = 128×128, field-of-view = 2 cm × 2 cm, slice thickness =1 mm and number of slices = 11 (contiguous). Java based DTI analysis software was used for the generation of FA (Fractional Anisotropy) and MD (Mean Diffusivity) maps (4). Regions of interest (ROI)s were placed on somato-sensory cortex (SMC), corpus callosum (CC), hippocampus (Hip), cerebral peduncle (CP), cingulum (CG), and caudate-putamen (CUP) regions bilaterally. 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 measures among different timepoints.

Results: At any timepoint, no abnormalities were observed in any group on anatomical images. A significantly decreased FA values were observed at both day3 and day5 in hippocampus in fractionated 8 gy cranial irradiation group compared to controls (**fig.1**). In single dose cranial irradiation group significantly decreased FA values were observed in hippocampus at all the three timepoints (1, 3 and 5 day after irradiation) compared to controls. In single dose irradiated group, a significantly decreased FA values were observed at day5 in CUP and CC region compared to controls. Significantly reduced MD values were observed in SMC and CUP regions in single dose irradiated group compared to controls at day5.

Table: A summary of group mean ± SD of FA collected from brain of fractionated group, single irradiation group, and controls.

Regions	control[a]	Fractionated group[b]			Single dose irradiation group[c]			P values
		1 D	3 D	5 D	1 D	3 D	5 D	
SMC	0.24±0.03	0.22±0.05	0.21±0.03	0.19±0.03	021.±0.03	0.24±0.03	0.23±0.03	Pa-b5=0.16,
CC	0.53±0.02	0.51±0.05	0.48±0.05	0.50±0.09	0.51±0.01	0.48±0.04	054.±0.02	Pa-c5=0.03
Hip	0.16±0.03	0.13±0.02	0.11±0.02	0.10±0.02	0.12±0.01	0.10±0.01	0.09±0.02	Pa-b3<0.001,Pa-b5<0.001,Pa-c1<0.001,Pa-c3<0.001,Pa-c3<0.001
CP	0.56±0.06	0.52±0.04	0.51±0.04	0.52±0.05	0.55±0.03	0.54±0.04	0.56±0.05	--
CG	0.41±0.08	0.35±0.03	0.37±0.06	0.35±0.03	0.34±0.02	0.37±0.03	0.37±0.02	--
CUP	0.18±0.04	0.19±0.06	0.16±0.03	0.12±0.03	0.14±0.01	0.15±0.03	0.12±0.02	Pa-c5=0.01

Note: SMC=sensory-motor cortex, cc=corpus callosum, Hip = hippocampus, CP=cerebral peduncle, CG=cingulum, CUP=caudate-putamen.

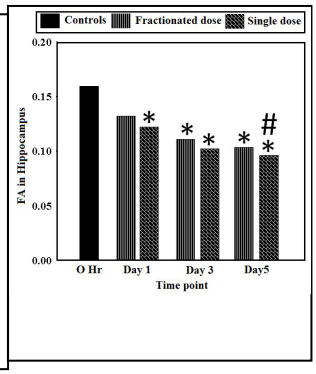

Fig. 1: Bars showing the FA values in hippocampus region at different time points in controls, fractionated group and single dose irradiation. * denotes significant difference with controls. # denotes significant difference with day1.

Discussion: In our longitudinal study we observed decreased FA values in hippocampus region in both fractionated dose group and single dose group at day3 and day5 after irradiation compared to controls. In addition, single dose irradiation group also showed decreased FA in CC and CUP regions at the time of day5 after irradiation compared with controls. With regards to fractionated therapy, our results showed that the single dose (8 Gy) induce earlier and more severe changes in the WM than the fractionated dose, and these differences could be reflected by the magnitude of change in FA values to some extent.

Some studies have found that reactive astrogliosis is a predominant histologic change as early as 3 to 24 hours postradiation until 24 weeks postradiation (5). Our observations of FA change in brain parenchyma after radiation injury can be explained by reactive astrogliosis. Though the mechanism is not clear about how reactive astrogliosis affects DTI indices, there is evidence from animal experiments measuring the extracellular space in brain injury models that diffusion is reduced in astrogliosis (6). We speculate that the reactive gliosis in acute phase of radiation injury is a probable cause of reduced FA in both fractionated group as well as single dose group. The hippocampus is one of the structures of the CNS where neurogenesis continues after birth. Radiation at much lower doses than that needed to injure the more resistant post-mitotic neurons and glia of the brain has been found to affect these highly proliferative progenitors severely. Our results demonstrate radiation induced microstructural changes in brain parenchyma even in case of fractionated dose during acute phase even before conventional MRI.

Conclusion: The present study indicated changes in FA values in different regions of brain associated with microstructural changes in brain parenchyma after fractionated and single whole brain radiation.

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