1H Magnetic Resonance Spectroscopy Reports Osmotic Dysregulation in Brain during Whole Body Radiation Induced Radiation Sickness

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Introduction: Moderate dose radiation exposure (1-10Gy) occurs due to radiation accident or during course of radiation therapy. In acute radiation syndrome, it is generally assumed that adult nervous tissue is highly radio resistant and is only affected at dose of 20-30 Gy. In reality, the CNS is radiosensitive in terms of functional criteria (brain electrical activity and neurochemical metabolism) for doses as low as a few grays. Few recent studies have shown immunogenic and histological changes in different brain regions after whole body irradiation. Furthermore, it has been suggested that radiation could activate reactive processes that generate inflammatory reactions in the CNS (Gourmelon et al, 2005). Therefore, the study was proposed to identify radiation induced early biochemical changes after whole body γ radiation exposure in mice model using in vivo MR spectroscopy.

Aim of the Study: To identify radiation induced biochemical changes in brain after exposure to different dose of whole body γ radiation in mice model using in vivo MR spectroscopy.

Materials and Methods: Male strain A Mice (n = 6 in each group) of 10 weeks of age were exposed to a radiation dose of 3, 5 and 8 Gy through Tele ^{60}Co irradiation facility unit gamma irradiation facility (Bhabhatron II) with source operating at 2.496 Gy/min. Controls (n = 6) were sham irradiated. MRI and MRS experiments were carried out at different time points of early phase post irradiation i.e day 1, 3, 5 and 10. All animal handling and experimental protocols conformed to the guidelines stipulated by the institutional animal ethical committee. All MRI/MRS experiments were performed on anaesthetised animals (i.p., xylazine (10mg/kg BW) and ketamine (80mg/kg BW)) at 7T on a Bruker Biospec with 30 cm bore magnet and B-GA20S gradient system. Radio frequency (RF) excitation was accomplished with 72-mm inner diameter (ID) linear birdcage coil and phase array coil for mouse head was used for signal reception. A rapid acquisition with relaxation enhancement (RARE) sequence (TE= 26 msec, TR=2500 msec) was used to acquire high resolution T2 weighted images of the mouse brain for positioning of the spectroscopic voxel. The MRS voxel was localised in the cortex-hippocampus region of mouse brain (1.5 x 3.5 x 3.0 mm^3; 15.75µl). Local field homogeneity was optimised using FASTMAP sequence. A water line width of each spectrum was within 15 Hz. Water signal was suppressed by VAPOR sequence before acquiring the spectra. ^{1}H PRESS (Point Resolved Spectroscopy) sequence (spectral width = 4006.41 Hz, data points =2048, averages= 512), TR (2500) and TE (20 msec) with total acquisition time of 21.33 mins was used for acquisition of MR spectra. MRS raw data (FID) was processed using LC model for quantitation. The data for each metabolite was tested for homogeneity of variances and one way ANOVA was used to compare means.

Result and Discussion: Quantitative analysis of 1H MRS using LCModel reported N acetyl aspartate (NAA), glutamine + glutamate (glx), tauirne (tau), myo-inositol (mI) and choline + glycerophosphocholine (tCh) metabolites after normalisation with total creatinine at different time points post irradiation in all three irradiated groups compared to controls. The results explained changes in mI and tau levels only in animals irradiated with 5 and 8 Gy (Figure 1). There was no significant difference in any of the metabolites in low dose (3Gy) group. Myo-inositol, taurine and other organic osmolytes present in brain together are proposed as a part of the volume regulation process (Flogel et. 1995; Kimelberg 1991). It is hypothesised that radiation induced injury is driven in part, via increased oxidative stress through generation of free radicals in brain. Earlier studies have anticipated free radical induced impairment in membranal ion pumps as a possible cause of dysregulation of osmotic balance in astrocytes (Brand et al, 1999; Kimelberg HK 1991). Changes observed in cortex-hippocampus area in our study could be a result of dysregulation of osmotic control in astrocytes or other glial cells due to radiation induced oxidative stress. Dose dependent changes were observed in irradiated groups. In case of 8 Gy, the changes in both tau and mI started appearing day 3 onwards post irradiation and persisted till day 10. Whereas, in case of 5 Gy, at day 5 post irradiation, both mI and tau levels were decreased compared to controls. Later on, mI level was recovered but tau continued to decrease till day 10 post irradiation. Reverting back of mI level to normal at day 10 in case of 5 Gy indicated some reversible change in this group and pointed out the ability of anti oxidant system of body to cope up with radiation induced oxidative stress as time progressed in case of low or intermediate dose. Repeated measure one way ANOVA also observed significant differences in both mI and tau levels between post and pre-irradiation level in case of 8 Gy dose group. Time dependent decrease in mI/Cr+PCr and tau/Cr+PCr values were detected day 3 onwards and continued to decrease till day 10 post-irradiation (Figure 2). Furthermore, using combination of metabolite (tau and mI) identified in this study, it was possible to predict whether brain injury has occurred after radiation exposure. For example, plot of taurine as a function of myo-inositol was able to segregate in two phenotypes for 8 and 5 Gy from control at day 5; whereas 5 Gy dose group was not distinguishable from control at day 10 post irradiation (Figure 3).

Conclusion: ^{1}H MRS has provided a unique opportunity to acquire brain metabolite information after whole body irradiation. The progressive decrease in taurine and mI with increasing time duration post-irradiation reflects osmotic dysregulation in cortex-hippocampus region. In our knowledge, this is the first longitudinal *in vivo* study that has demonstrated metabolic changes in response to whole body radiation and has demonstrated osmotic dysregulation after radiation exposure.

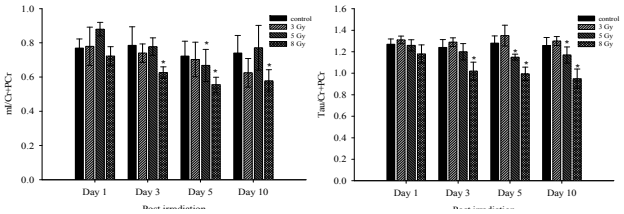

Figure 1: Temporal changes in mI/Cr+PCr and tau/Cr+PCr ratios in irradiated group compared to controls.

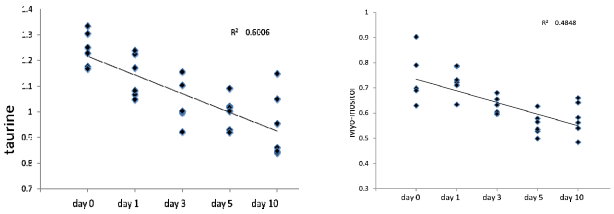

Figure 2: The relationship between taurine and myo-inositol levels and time duration post irradiation. The solid lines are the best linear fits through regression analysis.

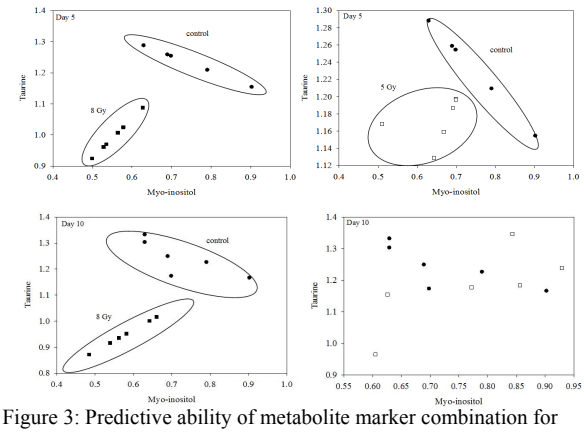

Figure 3: Predictive ability of metabolite marker combination for identification of phenotypes for radiation exposure

References:
1. Gourmelon et al. (2005) British Journal of Radiology 27:62-68
2. Flogel et al. (1995) Neurochemistry Research 20:793-802
3.Brand et al. (2005) Journal of Neuroscience Research 58: 576–585
4.Kimelberg HK (1991) Advances in comparative and environmental physiology volume 9: and osmolarity control in animal cells. Berlin, Heidelberg: Springer-Verlag 81-117.