Whole body radiation exposure induced neuometabolic alteration in murine brain: An In-vivo approach of 1H and 31P MR Spectroscopy

Ahmad Raza Khan[1], Poonam Rana[1], Hemanth BS Kumar[1], Shilpi Modi[1], Saleem javed[2], and Subash Khushu[1]

[1]NMR Research Centre, Institute of nuclear medicine and allied sciences, Delhi, Delhi, India, [2]Department of Biochemistry, Jamia hamdard, New Delhi, Delhi, India

Introduction: In the last few decades nuclear material are extensively used for power generation, medicine and industry besides nuclear armaments. This leads to the intentional or unintentional ionizing radiation exposure to the large population, which affects badly on human health as cognitive decline, cancer, hormonal disturbances and many more. In this scenario, high-throughput noninvasive studies are required to develop multiparameteric approach with strong prognostic capabilities which could provide help in subsequent medical management and treatment decisions. In the last two decade due to the technical advancement a number of tools and techniques are added in the panoply of MRI, which makes this technique more robust and applicative in disease and diagnosis, especially in case of brain. MRI based ^{1}H and ^{31}P MRS are able to detect metabolic abnormalities non-invasively due to functional impairment in normal appearing brain tissue. The present study reveals altered neurochemical status of hippocampus through ^{1}H MR spectroscopy and status of whole brain energy metabolites through ^{31}P MR spectroscopy. ^{1}H and ^{31}P MRS reveal altered metabolic profile of hippocampus and disturbed energy status due to whole body radiation 3 Gy and 5 Gy dose of radiation exposure.

Aim and Objective: A time dependent alteration of neurometabolites in murine brain due to non-lethal dose of whole body radiation exposure.

Material & Methods: Male strain 'A' mice of 10-12 weeks of age (n = 5 each) were exposed to radiation dose of 3 and 5 Gy from ^{60}Co source in a Telecobalt irradiation facility (Bhabhatrom-II) operating at a dose rate of 2.29 Gy/min and controls (n = 5) were sham irradiated. MRI and MRS experiments were carried out at day 1, 10, and day 30 post irradiation time points. All animal handling and experimental protocols were conformed to the guidelines specified by the institutional animal ethical committee. All MRI experiments were performed on anaesthetized animals (i.p., ketamine+ xylazine (80+ 10mg/kg BW) on 7T 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 ^{1}H MRS signal reception. The ^{1}H MRS voxel was localised in the right cortico-hippocampus region of mouse brain (1.5 x 3.5 x 3.0 mm^3; 15.75µl) each (Fig: 1). The local field homogeneity was optimized by adjustment of first and second order shim currents using FASTMAP sequence. The field homogeneity of voxel typically resulted in water line width of ≥15 Hz in live mouse brain. The water signal was suppressed by variable power RF pulses with optimized relaxation delay (VAPOR). Eddy current compensation and static magnetic field drift correction were applied during data acquisitions. Outer volume suppression (OVS) combined with a PRESS (Point Resolved Spectroscopy) sequence with spectral width of 4006.41 Hz, 2048 data points, 512 averages, 2500 ms TR and 20 ms TE with total acquisition time of 21 minutes was used for acquiring the ^{1}H MR spectra. The ^{31}P MR spectra were obtained from whole brain using a dual tuned surface coil (diameter 20 mm), for ^{1}H and ^{31}P frequencies at 300 and 121.49 MHz respectively. After shimming, voxel was positioned (4.0 x 6.0 x7.0 mm^3) 168 µl each (Fig: 3). ^{31}P MR spectra was acquired using image selected intra voxel (ISIS) localized spectroscopy having 8 acquisitions in a scan with spectral width of 7911.0 Hz, 2048 data points, 3.5 sec TR, 128 averages, and 2 dummy averages for a total scan time of 60 minutes and 40 sec. The carrier frequency of the adiabatic excitation pulse was positioned at the frequency of PCr i.e PCr acts as an internal reference to the spectrum (0.00 ppm). ^{1}H MRS data (FID) was processed using LC model and ^{31}P MRS was processed with AMARES algorithm of jMRUI software package for quantification of metabolites. The data for each metabolite was tested for homogeneity of variances and one way ANOVA was used to compare means.

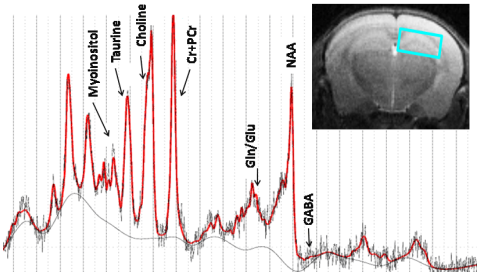

Figure: 1 Representative ^{1}H MR Spectra from hippocampus brain.

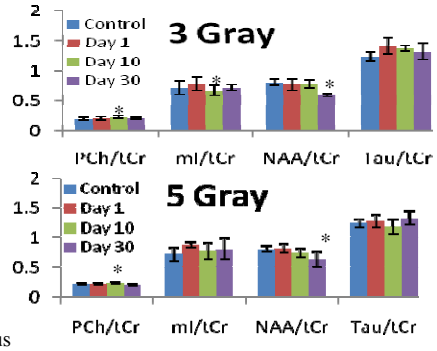

Figure:2 Bargraph of quantified neurometabolites

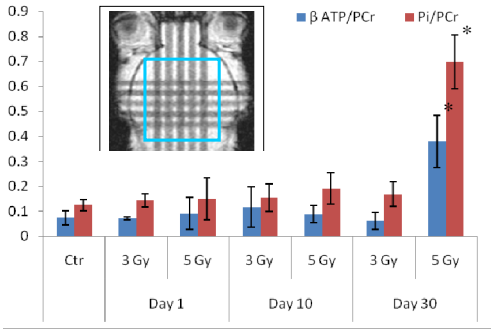

Figure:3 Bargraph of quantified phosphometabolites from ^{31}P MR Spectra representative voxel of mice

Result and Discussion: Quantification of in-vivo metabolites viz. Phosphocholine (PCh), myo-inositol (mI), N acetyl-aspartate (NAA) and tauirne (tau) were performed after normalisation with total creatinine at different time points post irradiation in both of the irradiated groups compared to control using LCModel. The result showed a persistent decrease in NAA level in both the irradiated group after radiation exposure (Fig :2), however decrease in NAA is significant only on day 30 post irradiation, which is in accordance with the earlier studies of radiation exposure where neuronal loss appeared at later time points of the study (Tofilon and Fike 2000). Taurine and myoinositol were not altered at later time point of the study; however they altered significantly at acute stage of radiation exposure, discussed in our previous study by Rana et al. 2012. These metabolites might be altered due to free radical induced oxidative damage produced by radiation exposure leading to cellular abnormal cell to cell interaction and cellular swelling. Free radicals also affect the mitochondrial function by altering Na^+-K^+-ATPase, Ca^{2+}-ATPase and the Na^+-Ca^{2+} exchangers, this might results in enhanced energy demand after radiation stress. ^{31}P MR Spectroscopy based metabolites play an important role in energy and membrane metabolism and alterations in these phosphometabolites of brain have been reported in various neurodevelopment disorder (3). The present study reported an increase in β ATP/PCr and Pi/PCr in whole brain after irradiation in 5 Gy dose group at later time point of the study, however no significant alteration were observed in 3 Gy dose group at any time point of the study.

Conclusion: ^{1}H and ^{31} P MRS based methodology is able to detect functional abnormalities in brain metabolism in normal appearing brain tissue. These finding may be helpful for radiation exposure cases such as radiation workers and victims of radiological accident.

Reference: (1) Tofilon & Fike Rad Res 2000; 357- 370; (2). Rana, P. et al. Int. . Rad. Biol. 2012 doi:10.3109/09553002.2013.734944; (3) Forlenza et al. Psychopharmacol. 2005; 180, 359-365. 2005.